

nature

28 July 1977

Peking exacts too high a price

THE cathedral city of Durham in the north of England is to be the site of an unusual battle in a week's time. For the question of the representation of the People's Republic of China in an international scientific union is to be put to the test, and, as in the international political bodies, the precondition of membership is to be the expulsion of Taiwan. There is by no means universal agreement that this is a worthy action.

Last year the International Union of the Geological Sciences (IUGS), meeting in Sydney, Australia, voted that the Academia Sinica of Taipei was not a valid body to be affiliated to IUGS, and went on to accept the Academia Sinica of Peking's application for membership. The Australian government had made its own small contribution to this decision by refusing visas to the Taiwanese delegates. The International Council of Scientific Unions (ICSU) didn't think much of all this and told IUGS to look into ways in which Taiwan could be reinstated.

The next union in line for a conditional application by the People's Republic of China was the International Union of Geodesy and Geophysics (IUGG), and its secretary-general, Professor P. Melchior of Brussels, is convening a council meeting, followed by an extraordinary general assembly (for the purposes of ratification) to coincide with a routine scientific gathering in Durham. Professor Melchior, who knows the People's Republic of China well, has a statement by the IUGG Bureau to fortify him. It is generally expected that this statement will assert that Taiwan is a province of the People's Republic, and so only Peking should represent China, and Taiwan's representation should cease.

Very few delegates would question the need for the People's Republic to be absorbed into international bodies, but it is to be hoped that many will balk at the price. Gone, clearly, are the days when Taiwan could claim to represent mainland China, but equally, the day is still far off when Peking can claim *de facto* to be able to represent Taiwan, which indisputably runs and finances its own independent scientific activities. If Rhodesia can be a full member of IUGG alongside the United Kingdom, has the People's Republic, not yet even a member, strong grounds to dictate that the academy of an irritant government, running a quite respectable programme in geophysics, should be wiped

off the international scene? Certainly such actions don't seem to match up in any way to the grand talk of the universality of science and its freedom from politics.

Many countries have already filed postal votes without bothering to hear the debate in Durham. Others are coming with instructions to admit the People's Republic, probably for the reason that they would rather be friendly with Peking than Taipei. So it is generally expected that Peking will have its way. Against all this the United Kingdom is to raise the question of universality and various constitutional issues—the latter a little unwillingly, because IUGG's constitution was not fashioned with such squabbles in mind and so could simply give legalistic nit-pickers a field day. The United States is expected to follow the same tack and will warn that back home the government won't like this sell-out of Taiwan by scientists and will look rather carefully and with due deliberation at the annual subscription that IUGG levies.

If the vote goes Peking's way, ICSU must, in logic, take at least as firm a line as it did with the geologists—firmer, preferably, because IUGG cannot claim to be ignorant of ICSU's views. And IUGG will have to face up to a problem in 1979 when it plans to hold a large meeting in Melbourne, Australia. ICSU rules now state that if any host country refuses visas to *bona fide* scientists wishing to attend a meeting, the relevant union must put pressure on that country. The pressure would presumably be in the form of a threat to move the meeting elsewhere or even cancel it. On the past form, Australia will turn away scientists from Taiwan. (IUGG may expel Taiwan, but it still recognises the right of qualified scientists from 'every part of the world' to participate as individuals in its meetings.) So IUGG must soon make its mind up not just over Taiwan's membership but also on some longer term consequences.

"If the international unions didn't exist, they wouldn't need to be invented", said one scientist last week who has had substantial experience with them. Even though they clearly serve a valuable purpose in helping a few scientists to travel and participate in international endeavours, the politicking that the People's Republic of China is trying to import could well seriously diminish what value the unions do possess. □



Cancer prevention: a question of priorities

Ernst L. Wynder of the American Health Foundation argues for the virtues of preventive medicine in combating cancer

RECENTLY, two independent studies have considered the relative contribution of environmental factors to the incidence of cancer*. Their common conclusion was that cancer is not an inevitable consequence of living, and that most cancers appear to be related to life-style factors. Estimates on the contribution of lifestyle to cancer incidence generally parallel each other (see below). The unanswered question is how we should react to our failure to curb those environmental factors that cause the continued high risk for many cancers.

As long ago as 1950, epidemiologic evidence established the causative association between cigarette smoking and lung cancer. Twenty-seven years later, tobacco usage still accounts for more than one-third of all male cancer deaths in the United States, in Britain and in other countries. Little did we expect that man's illusion of immortality would foster public apathy toward health messages related to smoking; nor did we foresee the unwillingness and inability of the medical establishment to assist significantly the motivation of the public to modify health behaviour. The opposition of commercial and agricultural interest, of course, was to be expected.

With the impact of these countervailing forces now so recognisable, the measure of success of a prevention-oriented approach to health maintenance seems to be inversely proportional to the responsibility one must assume for one's behaviour. It is thus far easier to launch a campaign directed against occupational and iatrogenic cancers than it is to infringe upon personal behaviour since, with the former, one can marshal the energy of government, the media and unions. It is no wonder that Doll regards the efforts necessary to solve the problem of nutrition-related cancers as an even more difficult task.

*Doll, R., *Nature* 265, 589-596; Wynder, E. L. and Gori, G. B., *J. Natn. Cancer Inst.* 58, 825-832.

The identification and subsequent reduction of environmentally induced cancers requires a multidisciplinary undertaking, ranging from molecular biology to public health. Just as Comprehensive Clinical Cancer Centres can more effectively prescribe treatment regimens for cancer patients, a multi-faceted approach to environmental cancers would provide a vehicle for researchers to work in tandem on issues related to environmental cancers. The creation of Interdisciplinary Centres for Cancer Prevention is now called for.

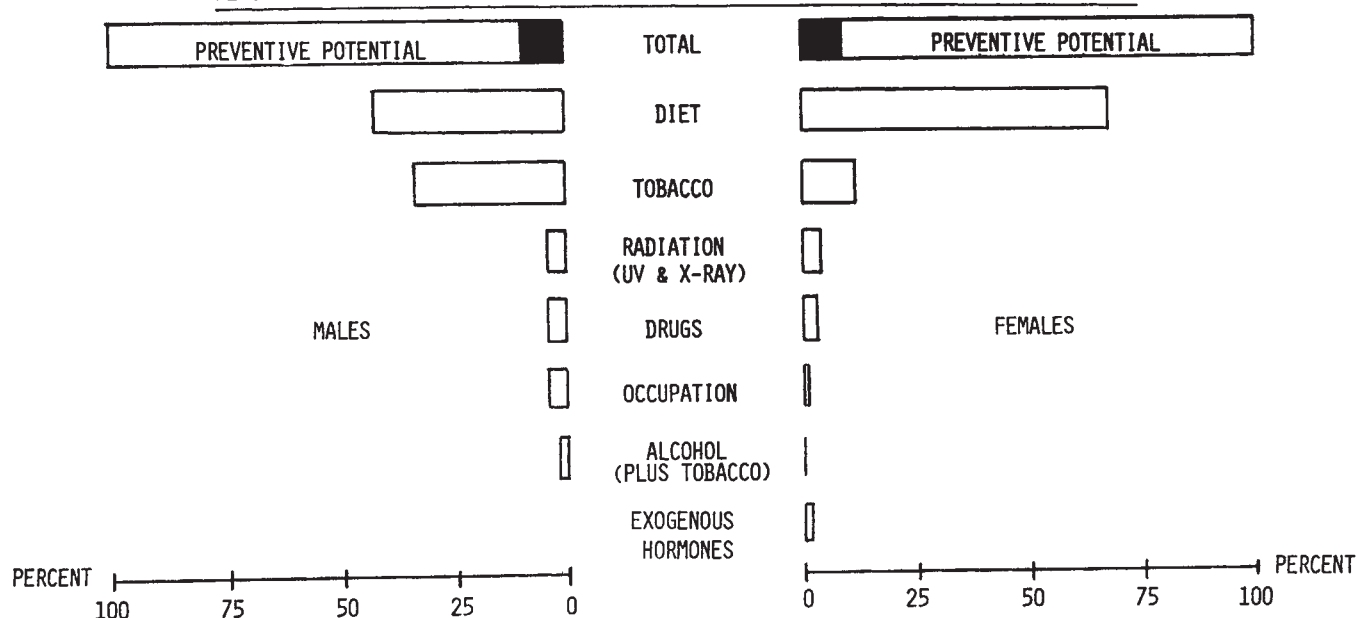
The cross-fertilisation between epidemiologists, biologists, chemists and public health professionals has already proved constructive in focusing, directing and applying research efforts. For these efforts to succeed, however, the broad support of our academic and government institutions, and especially of the scientific community, is required.

Endeavours in the field of prevention are currently receiving only a fraction of available government or private funding, with the possible exception of investigations on industrial carcinogens. Economic considerations may force a change. As disease care costs continue to rise, prevention will necessarily attract greater attention to avoid bankrupting the existing medical care delivery system.

It has been said that those who do not learn from history are condemned to relive it. The lessons to be learned from scurvy, smallpox and cholera can be repeated for most cancers, as well as for other chronic conditions. Eradication of a disease is not dependent upon fully comprehending the disease mechanism. This applies to cancer prevention as well.

The question remains whether we will accept these lessons from history, particularly when personal and societal 'sacrifices' are demanded. A patient dying from a self-inflicted cancer would, in retrospect, certainly answer with an unqualified "yes." But what of the rest of us looking toward the future? The development and support of Interdisciplinary Centres for Cancer Prevention could help us reach the proper perspective on the values of preventive medicine. It could also draw us closer to the day when what we know to be avoidable disease will no longer plague the health and economic fibres of ours and future generations. □

PERCENT OF CANCER INCIDENCE ATTRIBUTABLE TO SPECIFIC ENVIRONMENTAL FACTORS - USA



Soviet genetics: new controversy

Zhores Medvedev reviews the development of human genetics in the Soviet Union since Lysenko

T. D. LYSENKO dominated Soviet genetics and biology for more than 25 years. In 1965, when his domination was over, genuine science was quickly restored to research and education. It was generally assumed that his political and ideological interference in the nature of genetic problems had been completely wrong and harmful. The notions of a 'Soviet' and a 'bourgeois' natural science had been almost forgotten. In spite of Lysenko's long 'dictatorship', there were still quite a few prominent, enthusiastic geneticists of the older generation who could build up research again, write new textbooks for schools and universities, and support and teach the younger generation.

For human genetics, however, the position was much more bleak. The subject was declared racist and was completely destroyed in 1936–38. Most of its prominent representatives were arrested, and not one of them was found alive when Khrushchev dismantled the GULAG camp-prison system in 1956. The liquidation of Soviet human geneticists during the pre-war period explains why medical and particularly human genetics lagged behind in the rapid development of genetics after 1965.

The problems of human genetics were discussed only very briefly. In the first Soviet textbook on general genetics, written by Professor M. E. Lobashov and published in 1967, attention focused on the pattern of human chromosomes and immunogenetics. In only one chapter did Lobashov really repeat the old 'clichés' on the bourgeoisie's misuse of human genetics. Through human genetics, he claimed, this had tried to "justify the exploitation of one class by another, antagonism between different nations and creation of the racist theories". Lobashov also made some very positive statements about eugenics and considered that previous errors in this branch of human genetics did not necessarily indicate that it could not be developed into a useful field of research. Lobashov's view was that individuals in a human population, although equal in the social sense, have different genotypes and far from identical intellectual possibilities. These inherited differences had to be taken into consideration by educational training programmes so that the individual's

potential could be released and developed.

At the same time, I. T. Frolov, philosopher and senior official of the Department of Science of the Central Committee of the Communist Party of the USSR, published a small booklet entitled *Methodological Problems of Genetics*. His main points were designed to prove that the collapse of Lysenko's pseudoscience would not in any way diminish the importance and validity of Marxist dialectic-materialist philosophy for biology and natural sciences in general. Lysenko simply did not use this philosophy correctly; he falsified and distorted both biology and dialectical materialism.

With his philosophical criticism of Lysenko's ideas, Frolov attempted to present the Marxist background to modern classical genetics, biochemical genetics and evolution. Human genetics were not mentioned in his essay at all, as if they were not part of the problem; the rehabilitation of genetics stopped short, neither ideologists nor scientists being able to analyse human genetics. Nobody wanted just to acquire knowledge from abroad, but all the roots of Soviet human genetics had been completely destroyed, and as yet nothing could appear from the rubble.

Development inevitable

The logic of science and the practical needs of medicine, however, made the development of human and medical genetics in the USSR inevitable. The Academy of Medical Sciences started to consider establishing a research institute in the field, and the leading figures of genetics and biology, none of whom had real practical knowledge of human or medical genetics, started discussing their possible task.

Initially, the discussions were very peaceful and pragmatic with nobody, quite understandably, wanting to take the lead. Among those taking part, Academician Nikolai Dubinin, Director of the newly-established Institute of General Genetics, was considered to be the main authority. His expertise lay in the field of *Drosophila* genetics. The next most prominent but most popular of the geneticists was Academician Boris Astraurov. He was elected the first President of the Society of Geneticists of the Soviet Union, which was established in 1966, and an expert on silk worm genetics. He established the Institute of Developmental Biology which excluded research on human genetics.

Of the others actively involved in the discussions, Professor Dmitry Beliaev, Director of Novosibirsk Institute of Cytology and Genetics, had experimented in the genetics of rabbits and some rodents. The man most excited about human genetics, Dr Vladimir Effromson, was a silk worm geneticist whose very wide knowledge of medical genetics (he published the first Soviet book on medical genetics in 1963) was purely theoretical. The other members of the discussions came from similar backgrounds: Professor S. J. Alikhanian was Director of the Institute of Bacterial Genetics and Selection; A. A. Prokofieva-Belgovskaja and N. P. Bochkov were cytologists; N. P. Timofeev-Resovsky and R. L. Berg, drosophilists.

Although the project to establish the research institute on human genetics was generally supported, its organisation and priorities were not established in the meetings and discussions sponsored either by the Medical Academy or by the newly-created All-Union Society of Geneticists. The peaceful but not yet very productive discussions, however, were suddenly sharpened by Dubinin, who, like Frolov one year earlier, published a small popular book under the almost identical title, *Some Methodological Problems of Genetics*. The essay strongly politicised the discussion and opened up a new division among geneticists who only recently had represented a strong and friendly joint front against Lysenko.

Dubinin, introduced by the publisher as "a famous geneticist who had great influence on the development of Soviet and world genetics", started his essay in the usual demagogic style of his former Lysenkoist opponents. The methodological principles of the modern natural sciences, he claimed, had been founded by Lenin in his work *Materialism and Empirio-criticism* (1909). This had eternal importance for the further development of philosophy and all natural sciences, genetics in particular. After a short description of the history of modern genetics, with more than generous attention to Dubinin's own contributions and frequent quotations from Marx, Engels and Lenin, Dubinin unexpectedly made a statement on human genetics, differing little from Lysenko's approach to the field.

"In research on human genetics the main task is to realise that the human race during its development excluded itself from the evolution of other animals . . . Now social factors only—class struggle, industrial, cultural and scientific progress—determine human evolution. Anthropogenesis is completed and ended by the appearance of consciousness. Now social factors are primary and biological factors secondary". (p. 60).

At first this statement did not look

out of place. Nobody considered Dubinin an expert on human genetics and his colleagues and friends tried to explain to him that genetic laws and principles apply equally to all animals and that man is no exception. Very quickly, however, it became clear that the statement quoted above was not accidental; it was part of a developed system of views which recognised the human race as a qualitatively different, new and final stage of animal evolution.

Two years later, in 1970, Dubinin published another book for the general reader called *Genetics and Human Future*. By this time his ideas about the qualitative biological gap between man and other animals had developed. He tried to show that genetic make-up in humans bears no relation to the formation of personality. According to Dubinin's theory, human development from birth is determined by "social inheritance", everybody having potentially equal intellectual capabilities. Social inheritance is determined by the character of society itself and so far only the Communist societies can create the favourable conditions for the full development of human potential.

This book provoked more open discussion. Both sides tried to keep it outside serious academic journals, mostly because the discussion was based on opinions, not on facts or research. But both Astaurov and Effroimson wrote critical essays which were published in the popular literary magazine *Novy Mir* in 1971. Frolov, then Editor-in-Chief of the journal *Voprosy Filosofii* (Problems of Philosophy), organised a round-table discussion on human genetics, also sponsored by the Advisory Research Council, on the Problems of Philosophy of Nature at the Presidium of the Academy of Sciences of the USSR. It was clear from the discussion which was published in *Voprosy Filosofii* in 1972, that Dubinin's view received very little support.

Narrow objectives

The new Institute of Medical Genetics, which was finally established in 1971 within the Academy of Medical Sciences of the USSR, did not have the organisation to make the study of all aspects of human genetics possible. It was given narrow objectives and a comparatively young director, Nikolai Bochkov, a medical cytologist. Bochkov's appointment was supported by Dubinin who expected him to be an ally in the personal confrontations which now made up the discussion.

In 1973 Dubinin published *Vechnoe Dyizhenie* (Perpetual Motion), a rather self-promoting autobiography. Reviewed very widely and favourably in the official press, it distorted the factual history of Soviet genetics

and gave a very mild version of the Lysenko controversy. In it, though, Dubinin tried to reply to his critics and to formulate the main principles of his vision of human genetics.

"... The attempts to whitewash eugenics and to use for this purpose new genetic discoveries had been made by B. L. Astaurov, A. A. Neifakh and M. D. Golubovsky. V. P. Effroimson developed wrong views about genetic determination of intellectual and personal characteristics of man. This trend started to be an ideological danger. Again alien ideology tried to poison the clear sources of our science. I tried to make an uncompromised denunciation of this ideologically harmful attitude. Alas, I met a small, but well united group of opponents; B. L. Astaurov, S. M. Gershenson, S. I. Alikanian, D. K. Beliaev who defended their wrong position. This worried me and proved that some old geneticists do not understand Marxist ideas about human race and principles of society which is the only factor determining the intellectual specificity of man..." (p. 434).

'According to Dubinin, human development is determined by "social inheritance", which is determined by the character of society itself. Only the Communist societies can create the favourable conditions for the full development of human potential'

Dubinin also tried to develop the distinction between the genetic control of physiological and psychological characteristics of human individuals. Genes, according to his view, determine the programme of development, lifespan, some pathologies, expectancy of heart troubles, cancer and resistance to infections. The shape of nose and mouth, or height, are also inherited traits. However, personality and intellect, he claimed, depend not on genes but only on social environment and self-training; their possibilities are unlimited. As soon as man is able to think consciously, he isolates himself from the laws of biology. All "normal" persons are psychologically and mentally equal at birth and so there is no reason for taking the racist approach when talking about the quality of human genotypes with regard to intellect.

"... Let us remember the story of emigration from Russia of many representatives of bourgeois art, scientific and technological elite after the October Revolution. Some eugenicists considered this exodus as an irretrievable loss of valuable genes. But this was nonsense. New talents from grass roots through Revolution found their way to lead the country, the Party, to create new science, new art, to create the great Soviet Union!... This fact illustrates that the creation of personality does not depend

on genes, but is induced by the information of social environment." (p. 426).

In 1974 Academician Boris Astaurov died from a heart attack—a great loss for Soviet biology. His death was probably hastened by strong political pressures induced by the defection of one of the senior research scientists from his institute. As director, Astaurov had recommended that this good research worker present a paper at an international conference in Italy. The man asked for political asylum and did not return to the USSR. In such cases, the official bureaucracy makes directors personally responsible. Special Academy commissions started to 'investigate' (with active participation by Dubinin) all aspects of the ideological and moral atmosphere of Astaurov's institute. Some senior members of his staff were demoted or dismissed, and Astaurov's health suffered.

Not long before his death, Astaurov, as a member of the Editorial Board of a popular scientific journal *Priroda* (Nature), recommended that it publish a translation of the article "Man as a Biological Species" by Professor Ernest Mayr, a prominent American zoologist and population geneticist. The article did not ignore the social aspects of formation of human personality, but it considered all characteristics and qualities of individuals to be a result of the interaction of genotype and environmental and social influences.

Mayr's article provoked interesting discussion in *Priroda*; several anthropologists and philosophers supported his views. Dubinin did not join in the *Priroda* discussion, but instead attacked Mayr's article in a paper published in a philosophy journal where he insisted that all the spiritual aspects of human life are outside biology or genetics. He labelled Mayr's synthetic, social and biological approach as "reactionary" and "racist".

Reply published

The average publication time in Soviet journals, including popular scientific ones, is very long. Consequently a reply to Dubinin's article, written by D. K. Beliaev, was not published in *Priroda* until a year later. Beliaev, who in 1976 had already been appointed President of the 1978 International Genetic Congress in Moscow, was reluctant to acknowledge the seriousness of the controversy among the Soviet geneticists. He clearly explained, however, that he disagreed with Dubinin's simplistic explanations. Behaviour, he wrote, as well as many of man's intellectual abilities, certainly depend on social factors, teaching and training, but these factors act on a highly differentiated population and the main organ of mental activity—

the human brain—is not excluded from genetic laws and genetic diversity.

Dubinín published a new detailed article on the subject which appeared earlier this year in *Voprosy Filosofii*. It mostly repeated his earlier arguments, though it was more politicised. The ideas about the intellectual or spiritual aspects of human life became rather mystical, in spite of regular quotations from Marx, Engels and Lenin.

Through a complex of pseudo-scientific argumentation, Dubinín tried to state that the intellectual and ethical progress of *Homo sapiens humanis* is properly directed in one social system only. In any other social system there is no progressive evolution. Social systems create the real man after birth; the priority of genes before birth is not questioned. Good systems create good

persons, bad create bad. From Dubinín's autobiography it is clear that he considers himself a good man whose psychology and intellect has been developed under the influence of the best social systems. "I am thankful for my fate . . . I do not want to live another life . . ." He is happy. During his 70 years of life, he never discovered that 30 years of Stalinist terror were not good for the creation of *Homo sapiens humanis*.

There is, however, at least one important factor for securing the happiness of *Homo sapiens logic*—freedom to do research, whether genetic, social or political. Using his position as the director of the main Institute of General Genetics, his influence as Consultant on Biology for the Central Party Committee and his membership

of different commissions and groups, Dubinín is working hard to suppress by all possible means the development of genuine research in the field of human genetics in the USSR. Even now the Soviet Union is not involved in many important fields of research in human genetics. As part of medical science, human genetics now has modest conditions for research into several specially selected problems, mainly associated with health programmes. But as a part of general biology, evolution research or population genetics, human genetics does not really exist in the USSR.

In a great country with more than a hundred national minorities, several different races and many different cultural roots, human genetics is probably too hot a science to handle. □

ILL at ease

Judy Redfearn finds out about neutron research at the Institut Laue-Langevin and talks to its director

NEUTRONS have long been recognised as valuable tools for research. Their importance is still growing as new techniques are enabling them to find new applications in different branches of science. One of the world centres for neutron research is the Institut Max von Laue-Paul Langevin (ILL) in Grenoble, France. It is jointly run by Britain, France and Germany, and this year, when it celebrates its tenth anniversary, has seen the appointment of a British scientist as its director.

He is Dr John White, a physical chemist from St John's College, Oxford, who took over from Professor R. Mössbauer, a German, last March. He faced a pressing problem after he took up his new post: what to do with spent fuel rods from the high flux reactor, the cornerstone of ILL's neutron facility. ILL has been assured of a fuel supply since President Carter lifted his ban on the export of enriched uranium, but the freeze on reprocessing caused some worries. Just last week, however, it was granted a licence to import 12 fuel elements into the USA for reprocessing at ERDA's Savannah River plant in Georgia.

But President Carter's policy is not the only factor which may influence ILL's future. The lifetime of the high flux reactor and the value of all ILL's facilities to its member states are now being assessed to determine the institute's long term prospects after 1982, the earliest date at which the three member countries could, under the

original agreement, revoke their commitment. An extensive study of the reactor has shown that it may yet have 20–25 years more life in it—"a tribute", says Dr White, "to the French and German engineers who built it". If further studies support this, a report on the institute's future, due in the autumn, should give a firm assurance that the reactor will continue operating into the 1980s.

How it will continue, however, is not yet certain. The report is expected to recommend whether or not it should go into a 'deuxième souffle'—a second stage involving the member states in another major investment to extend the facility's scope. Dr White believes that ILL will only survive into the 1980s if instruments and techniques are constantly up-dated. "At the moment we have got world leadership in this area—and we have got to keep it up."

A matter of satisfaction

ILL's future success will depend, as it always has, on how well it satisfies its visiting researchers. Unlike many other laboratories and research organisations, it is essentially a users' laboratory. Some 70% of the neutron-beam time is available for outside experiments, mainly from universities and institutes in the three member countries but also from many other parts of the world. Only 400 staff, of which 70 are PhD scientists, are employed under five-year contracts to keep instruments running. ILL's Scientific Council considers approximately 700 experimental pro-

posals each year; at its last meeting in March, it reported requests for twice as much experimental time as is available.

Experiments originating in the member countries are funded by the organisations, or Associates, which run the institute: the Science Research Council in Britain, the Gesellschaft für Kernforschung in West Germany, and the Commissariat à l'Energie Atomique and the Centre National de la Recherche Scientifique in France. France and Germany signed the first inter-governmental treaty giving the go-ahead for the building of ILL in 1967. It was not until 1973 that Britain, having abandoned an idea to build its own intense neutron source, joined in, paying a share of the original capital investment. Routine operation of the high flux reactor, which cost FF240 million of the FF335 million spent on building the whole institute, began in 1972. It has almost completed five years of successful operation and most of the instruments designed around it



ILL's director, John White

have now been working for 2½ years.

The high flux reactor is possibly the most intense continuous neutron source in the world at the moment. It operates at 57 MW and contains a single fuel element of 9 g of 90% enriched uranium. Because such highly enriched uranium is contained within a small reactor core, the fission process is concentrated into a small volume and the resulting neutron radiation is very intense.

"If the first unique feature of this reactor is its high flux density", says Dr White, "the second is the interesting and varied sources of neutrons which surround the core". Neutrons straight from the reactor are all slowed down to thermal velocities as they pass through a vessel of deuterium at 300 K. Those emerging from a particular part of the reactor are then speeded up by a piece of hot graphite to form a hot source. Other neutrons pass through liquid deuterium at 25 K and are slowed down even further to form a cold source. But perhaps the most important feature of ILL's reactor is the great increase in experimental area created by conducting some of the thermal and cold neutrons through special conducting pipes away from the space surrounding the reactor, which is crowded with instruments.

It is the ways in which they interact with matter that have made neutrons valuable research tools. Four properties make slow neutrons particularly useful in the study of liquids and solids: their electrical neutrality, short wavelength, low energy and magnetic moment. Because they have no electric charge they can penetrate the atom's electron shell and interact with the nucleus. Their short wavelength—of the same order as the atomic diameter—means that they are scattered by a regular array of atoms in a crystal to form a neutron diffraction pattern. Their low energy—of the same order as the quanta of vibrational energy of the scattering sample—means the dynamics of solids and liquids can be studied by

measuring speed changes in the scattered neutrons. So called magnetic scattering occurs when the magnetic moment of the neutron interacts with an atom's magnetic moment; it facilitates the study of the magnetic properties of solids and liquids.

Diversification

Physicists were aware of the value of the neutron's properties 20 years ago when they started using them for studies in solid state physics. But it is only over the past ten years that they have been used in chemistry; and in the past three to four years they have found applications in biology as well—last year saw the first international conference on neutrons in biology. Dr White says that ILL's curious instrumentation has helped it to make a unique contribution in some of the new areas of science, particularly in polymer chemistry, biology and nuclear physics. The structure of biological materials, for example, can often be revealed in the diffraction patterns of neutrons scattered from them.

The phenomenon which makes neutrons so potentially valuable in chemistry and biology is called 'contrast'. It can best be explained by analogy with light. A glass rod immersed in a liquid will be clearly visible provided that the refractive index of the glass differs from that of the liquid. If the liquid's refractive index is changed to be the same as that of the glass, the rod will seem to disappear. Similarly, a biological material can be immersed in a mixture of heavy and light water whose refractive index with respect to neutrons is the same as parts of the material. Those parts of the material will then make no contribution to the neutron diffraction pattern and will have been 'contrasted' out. This means, for example, that a neutron diffraction pattern could be formed from, say, the RNA molecules inside a virus, no contribution to the pattern being made by any other part of the virus.

Dr White believes that the recent branching out into broader areas of science has come from a happy interaction between the interest of the users of ILL and the instrumentation that was already there. Britain added to the creative atmosphere by bringing many problems to be solved; the French and German approach had always been to design instruments to find new ways of working with neutrons. The two approaches combined well to bring forward an expansion in the use of neutrons for research. As Dr White puts it, "The French are a bit Cartesian, they tend to think mathematically; the Germans are Leibnizian; and the British tend to be empirical". But he does feel that ILL gains

rather than suffers from the fusion of these three different scientific temperaments.

The institute is assured of a supply of fresh ideas not only because so many different nationalities work together, but also because there is a regular turnover of permanent staff. Although the average age of the staff, 34, means that ILL is a place for young people with good ideas, it does have one disadvantage. Each year the 2½–3% net growth in ILL's budget allowed for by the Associates is gobbled up as the predominantly young staff moves up the salary scale, leaving very little for building new instruments.

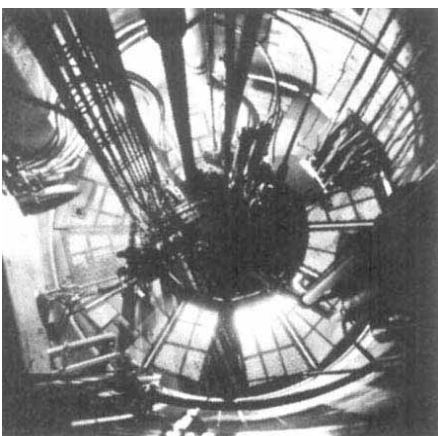
"At the moment we are only building one or so instruments a year, which is hopeless because the normal amortisation rate for such heavily used instruments as those at ILL is 10 years at the most. At the present rate of building, instruments will be 15 years old or more before they can be replaced". Somehow, ILL is hoping to find a way of subsidising its budget so that more instruments can be built.

Move under way

A move in this direction is already under way. Since March, Dr White's main scientific concern has been in establishing a source of ultra-cold neutrons which are just energetic enough to reach a height of 15 feet in the earth's gravitational field. They are totally internally reflected from quite common materials and so can easily be trapped for experimentation.

Perhaps the most exciting application they could have is in the field of neutron optics. Existing techniques for focusing neutrons, for example, are as crude as the techniques used by Newton 300 years ago to focus light. As yet there is no neutron lens but the ultra cold neutrons travel so slowly that they can be focused with a magnetic field and could be used to develop neutron-focusing techniques. Already a highly coherent neutron beam has been produced at ILL. It is by no means a neutron laser, but it does have some of a laser's properties which could be useful in neutron optic studies.

"The people who come to ILL have got particular problems that can be solved no other way than by neutrons", says Dr White. "Even five years ago, we wouldn't have believed that neutrons would have the sensitivity to detect the things they now do; but with the high flux and the specialised instruments at Grenoble, we have seen hitherto unobservable things". When the spallation neutron source—a pulsed source of very hot neutrons just approved in Britain—is built at the UK's Rutherford Laboratory, he claims that Europe will house the world's most sophisticated neutron facilities. □



ILL's high flux reactor:
view from the top

USA

Assessing the 'greenhouse effect'

A report on the climatic effects of energy consumption was made public last week. Colin Norman reports from Washington

IF THE world's burgeoning demand for energy is met by burning increasing amounts of fossil fuels, there could be significant changes in the Earth's climate, lasting at least a millennium, a committee of the National Academy of Sciences warned in a report made public last week. Though the report is hedged with uncertainties and qualifications, the committee reckons that if the use of fossil fuels continues to increase at present rates, average global temperature could rise by about 6°C over the next 200 years, with potentially dire consequences for agriculture and fisheries.

The report, prepared by a committee headed by Roger Revelle, a noted ecologist who holds joint appointments at Harvard and the University of California, constitutes the first major attempt to assess the validity and potential consequences of the so-called "greenhouse effect", which may result from loading the atmosphere with carbon dioxide by burning coal, oil and gas. The theory is that carbon dioxide in the atmosphere will absorb heat radiated from the Earth's surface, causing an increase in global temperature, which in turn could trigger a variety of climatic changes.

The greenhouse effect was first postulated several years ago, but the theory has been controversial and the magnitude of its potential impact has been the subject of considerable debate. The Academy committee acknowledges that the data supporting the theory are incomplete, but the evidence is nevertheless strong and "the implications warrant prompt attention". Though the committee looked into other potential climatic impacts of energy use, such as the direct addition of heat to the environment and increasing the amount of particulates spewed into the atmosphere, it found that the carbon dioxide effect is likely to be far the most significant and it "may be the primary limiting factor on energy production from fossil fuels over the next few centuries".

On the basis of a number of climatic models and several years of direct measurements, the committee calculates that the concentration of carbon dioxide in the atmosphere has increased by between 11% and 13% from the beginning of the industrial

revolution to the present. It may increase by 25% by the turn of the century, double by the year 2050 and reach between four and eight times its pre-industrial level in 200 years' time, if use of fossil fuels continues to grow.

The effect of such a rise in carbon dioxide concentration would be a 6°C increase in average global temperature over the next 200 years and temperatures at the poles would be expected to increase even more, the committee states. In addition, precipitation is likely to increase and there could be severe regional changes in weather patterns.

It is important, however, to note the basis for those predictions first, the projected increase in carbon dioxide concentration is based on the assumption that use of fossil fuels will continue to grow as the industrialised countries switch from oil and gas to coal to meet their energy needs. Another key assumption is that per capita energy consumption will increase and that population will rise to a level of 10 billion toward the end of the twenty-first century. Those projections could be greatly affected by policies to curb the environmental impact of strip mining, by deliberate attempts to convert to other energy sources, by efforts to curb energy demand, and by policies to restrict population growth. The report's con-

clusions are therefore essentially based on an extension of present trends.

The projections are, however, founded on some key measurements of carbon dioxide concentrations at sites in Hawaii and the South Pole over an 18-year period during the 1960s and early 1970s. The measurements, made by Charles D. Keeling of the University of California at San Diego, are the first positive confirmation that carbon dioxide levels are increasing. They indicate that carbon dioxide concentration increased by about 5% over the period, and they suggest that about 40% of the carbon dioxide released into the atmosphere had remained there. The committee concludes that about 40% of the carbon dioxide released during that period was taken up by the terrestrial biosphere and the remaining 20% was taken up by the oceans. The projections of a doubling of carbon dioxide concentration over the next century and a four-to-eight fold increase by the year 2050 are essentially an extension of those calculations and measurements.

The link between increasing carbon dioxide concentrations and increasing global temperature is based on a computer model of the Earth's climate which the committee acknowledges is "imperfect in a number of significant ways" but is "the most complete yet devised". The model predicts a 3°C increase in the temperature of the lower atmosphere for each doubling in carbon dioxide concentration, and a

TVA appointment

PRESIDENT CARTER last week gave a clear signal of his intention to set a new course for the giant Tennessee Valley Authority (TVA) by nominating S. David Freeman, an advocate of energy conservation and solar power, to fill a long-standing vacancy on TVA's board of directors. Freeman, who first came to prominence when he directed the Ford Foundation's multi-million dollar Energy Policy Project, is also expected to become chairman of the three-member board when the present incumbent, Aubrey Wagner, retires next year.

Established in the Great Depression as part of Franklin Roosevelt's New Deal, TVA has developed into the largest electric utility in the United States, serving an area the size of Great Britain. It was created primarily to raise the economic level of one of the poorest regions in the United States, but energy supplies

dominated operations. It is the largest single purchaser of strip-mined coal, and has had a bitter battle with the Environmental Protection Agency over its policy of dispersing pollutants from its power plants through tall smokestacks. TVA has also embarked on a massive nuclear programme, with plans to have as many as 17 nuclear reactors in operation by the mid-1980s.

Critics have contended that TVA's poor environmental record, and its relative neglect of alternative sources of energy, are a far cry from its original goal. Carter has echoed such complaints. Now he has indicated that he would like the authority to recapture some of the innovative spirit which predominated during its early years. Freeman is at present an assistant to James Schlesinger, Carter's chief energy adviser.

Colin Norman

7% rise in average precipitation.

The effects of such an increase in temperature could be serious. The committee notes that there would be a poleward shift in the world's agricultural zones, marked changes in the location and extent of arid and semi-arid zones, and potentially serious changes in regions of precipitation. For the north American breadbasket, for example, the effect could be to push the corn belt northwards off the productive soils of the midwest and on to poorer Canadian soils. The impact on the oceans could be even more severe. A warming of the surface layers could be expected, which would reduce vertical circulation, thereby reducing the biological productivity of the oceans because it would depress the circulation of nutrients. There would also be a significant shift in the location of the major fisheries.

A five-degree warming of the upper 1,000 m of ocean water would also raise the ocean level by simple expansion by about 1 m. Melting of sea ice would augment the rise in ocean level. As for the potential impact on the polar ice caps, the committee states that "in the present state of understanding, it is impossible to forecast what might happen to the Greenland and Antarctic ice caps". Since temperatures over the ice caps would be expected to remain below the freezing point, however, "melting at or near the surface of the ice probably would not occur". But increased precipitation associated with increased levels of carbon dioxide could lead to substantial increase in ice thickness, which in turn could cause severe stresses at the base of the ice caps and surges and slides of ice into the sea. "If these surges resulted in destruction of the west Antarctic ice cap, there might be a corresponding rise in sea level of about 5 m within 300 years", the committee notes.

The report does not consider in detail the potential impact of global warming on regional weather patterns, climatic variability and shifts in ocean currents because of the difficulty predicting such effects.

The committee's chief recommendation is that a major research effort should be instituted, on a worldwide basis, to address some of the major unknowns in the climatic models and measurements on which the projections are based. Such an effort could cost between \$20 and \$100 million a year, Revelle suggested last week.

The report's chief impact, however, is likely to be on discussions of energy policy. The committee notes that if the potential for climatic change is substantiated, it may be necessary to reverse the trend of consumption of fossil fuels. The committee adds: "In

Jupiter success

THANKS to an intense lobbying effort by space scientists, university administrators and Governor Jerry Brown of California, the Jupiter Orbiter Probe, a key space science project, has survived a strong challenge in Congress. The House of Representatives last week rejected a recommendation from its powerful appropriations committee that the project should be killed, and voted funds to begin building the spacecraft next year. Since the Senate had already approved the project, the House vote constitutes the final go-ahead.

The Congressional debate over the Jupiter project provides an object lesson in the politics of big science. It also demonstrates the power that the scientific community can muster when it unites behind a major project.

Long considered a top priority space science mission, the Jupiter Orbiter Probe will involve the launch in January 1982 of twin spacecraft to orbit Jupiter and send scientific instruments into the planet's atmosphere. The total cost is expected to be close to \$300 million. Though the project has been on the drawing board for several years, this is the first year that the National Aeronautics and Space Administration (NASA) has asked Congress for funds to begin building hardware.

NASA officials were not anticipating much opposition to the project from Congress, and they consequently received a shock earlier this year when a House appropriations subcommittee, chaired by Edward Boland of Massachusetts, recommended that all funds for the mission be deleted from NASA's budget. The subcommittee's action, which was later upheld by the full appropriations committee and the House itself, was based on the fact that NASA was also requesting funds to begin building the Large Space Telescope. The subcommittee said that it would be willing to sanction only one major space science project, and it opted for the telescope.

Once the subcommittee's vote against the project was made known, the scientific community mobilised to reverse the decision. A flood of letters and telephone calls descended on

members of the Senate appropriations committee in an attempt to persuade the Senate to keep funds for the Jupiter project in its version of the NASA budget bill. The lobbying was successful, and it was then widely expected that when differences between the House and Senate versions of the budget bill were ironed out by a House-Senate conference committee, the Jupiter project would emerge with sufficient money to meet the launch schedule. But further problems arose.

House members of the conference committee refused to compromise on the project, insisting that it must be dropped in order to provide more money for higher priority efforts. Senate conferees, similarly, insisted that the project be allowed to proceed. To break the deadlock, the matter was referred back to the House for a separate vote last week. The lobbying was at its most intense shortly before the House vote.

The Jupiter Orbiter Probe is the only planetary project up for approval, and NASA officials thus argued that if it were killed there would be a major hiatus in planetary research. Abandoning the project would result in breaking up teams of scientists and engineers who have been working on planetary programmes, and continuity from one project to another would be lost, NASA officials argued. Moreover, since there would not be another suitable launch window until 1987, denial of funds for the project this year would effectively put Jupiter out of reach for another five years.

The chief political asset for the project, however, was the fact that it will be based at the Jet Propulsion Laboratory in California, a state with a large Congressional delegation and considerable political muscle. Alarmed at the prospect of losing a \$300 million federal project, Governor Brown telephoned a number of Congressmen before last week's vote and urged them to support the project. As a result, the California delegation united with other supporters of the project, and the opposition of the appropriations subcommittee was easily overcome. The vote in the end was 131 to 280.

Colin Norman

the face of so much uncertainty regarding climatic change, it may be argued that the wisest attitude would be *laissez faire*. Unfortunately, it will take a millennium for the effects of a century

of fossil fuels to dissipate. If the decision is postponed until the impact of man-made climate changes has been felt, then, for all practical purposes, the die will already have been cast". □

BRITAIN

● Concorde supporters can take heart at the news that British Airways is to use the plane to fly twelve batches of generators of a newly-developed radiopharmaceutical across the Atlantic to waiting patients with lung complaints at Johns Hopkins Medical Unit, Baltimore, and the Washington Hospital Center. Doctors at Johns Hopkins took delivery of the first batch last week, and are reportedly delighted with it.

The new radiopharmaceutical, krypton-81m, helps doctors diagnose lung diseases. It was developed about two years ago by researchers at the Medical Research Council Cyclotron Unit at Hammersmith Hospital in London. It cannot be flown across the Atlantic subsonically because rubidium-81, the generator from which the krypton-81m is obtained, decays so quickly; it would be too weak to be used by the time it arrived. But Concorde cuts the transatlantic crossing time from 8 to about 3½ hours, and if twice the normal requirement of rubidium-81 is loaded onto the plane, sufficient radioactive strength remains after the journey for up to ten hours of clinical use.

It is krypton-81m's short half-life which makes it so useful. At 13 seconds, it is the shortest-lived radioisotope in medical use. The patient is positioned in front of a gamma camera and breathes in air containing a trace of the radioactive krypton which decays rapidly inside the lungs. The gamma camera records the regional arrival of air, enabling doctors to see immediately which parts of the lung are receiving no air. Previously only total lung volume could be measured, using long-lived isotopes.

At Hammersmith, krypton-81m has been used to monitor progress and response to different treatments. By itself it is not a good diagnostic technique, but in conjunction with standard perfusion techniques, which record blood supply to the lungs, it provides unique information for the diagnosis of such lung diseases as pulmonary embolism, asthma and chronic bronchitis.

● The good thing about the UK National Coal Board's (NCB) activities last year was the £27 million profit it made, said its chairman, Sir Derek Ezra, when the 1976-77 annual report was published last week. What wasn't so good, he said, was the fall in production and productivity.

This apparent paradox has arisen

three years after the introduction of Plan for Coal, a scheme set up in 1974 which would allow expanded exploration and research. Sir Derek puts 1976-77's profit down to achievements under the Plan as well as increased sales, a 10% increase in open-cast mining output and economies of operation.



The most notable short-term research success was the development of the in-seam miner, which won the Queen's Award for Technological Achievement. It has mechanised some manual tasks and has improved the working of face ends and the opening of new faces. Short-to-medium term mining research programmes aim at further automation. Fluidised bed combustors to improve coal utilisation are being developed for commercial use. They are already available on a small scale, but have yet to be developed for use in power stations.

The long term hopes for achievement in coal research, however, go hand in hand with expectations for coal in meeting future energy demands. If coal is to become a more important energy source as oil supplies dry up, the NCB must increase production, find new coalfields and develop ways to convert coal to chemical feedstocks, gas and liquid fuels. Under the long term plan, Plan 2000, research aims at completely automated mining, especially of deep or remote seams inaccessible to miners. One method enjoying moderate success is the removal of coal which has first been liquidised underground, and a multidisciplinary group has explored the long term possibilities of such methods with research establishments in the UK, USA and West Germany.

All in all the NCB spent £20.8 million on research in 1976-77, about 0.7% of gross turnover. Of the different research categories—mining, utilisation, medical and others—mining received the lion's share. The trend is expected to continue this year.

● Professor Thomas Symington, director of the Institute of Cancer Research (ICR), has announced his retirement two years before it was expected. The decision was made mainly on personal grounds and its suddenness will leave the Institute temporarily without a director. Professor Leonard Lamerton of the Institute's Biophysics Department will be director for the time being until a substantive director is appointed.

The Institute of Cancer Research, consisting of the Chester Beatty Research Institute in London, the Pollards Wood Research Station at Chalfont St. Giles in Buckinghamshire and several Departments on the Sutton, Surrey, site of the Royal Marsden Hospital, has an annual budget of about £3 million: half of this comes from the Medical Research Council (MRC), £650,000 from the Cancer Research Campaign and the residue from legacies' interest and grants from overseas. The ICR has had to face a fall in the MRC's block grant and the removal of a £200,000 allocation to the animal facilities. Symington told *Nature* that this was a temporary problem and had no influence on his decision to retire.

When Symington became director seven years ago, the Institute needed reorganisation to rid itself of its less effective research staff. The MRC insisted on converting much of its funding from a block grant into support of specific programmes and projects to be accounted for on a regular basis. By most accounts the Institute under Symington came to withstand that kind of scrutiny. He leaves wishing only that he could have unified the various parts of the Institute on the campus of a medical school.

● Pro-nuclear Labour Party activists could face a problem when the party gathers in Brighton for its annual conference in the first week of October. Of nine resolutions on energy, not one has a good word for nuclear power—and not one fails to mention nuclear power. Three resolutions appear on nuclear weapons, and just one on defence generally.

BRITAIN

Awkward decision

In an embarrassing and controversial decision, the UK Medical Research Council (MRC) last week agreed to "run down" its Demyelinating Diseases Unit in Newcastle. Chris Sherwell reports

ON THE face of it, the MRC's decision is in line with its overall policy regarding research units, namely that they last as long as the directors to which they are attached. A glance at the two most recent MRC annual reports, for example, shows a sizeable turnover, with two or three units a year appearing and more disbanding. The director of the Demyelinating Diseases Unit left over a year ago. So without a new director a run down and redeployment of the unit's 33 staff, if not closure altogether, was always on the cards.

The demonstration outside MRC headquarters on the day the decision was taken, however, suggested that this particular case was different. And so it is. Partly this is because a well organised and highly unionised staff at the unit was resisting the MRC,

an example of a phenomenon that the Council is only just beginning to accommodate. The more important reason is that the MRC has set such store by its multiple sclerosis (MS) research effort, of which the unit is a part. Not to be able to find a suitable director to take over the unit, which is the problem the MRC says it has faced, has made for many red faces. Some concerned lobbying from MS-related organisations has added to the controversy.

So too has the unit's stormy history, which finds its best expression in the circumstances surrounding the departure of E. L. Field as director in 1973. His controversial work is still debated, some say partly because the failed efforts to repeat his work have never been published. Henry Wisniewski became director in October 1974 but left in June last year for what were described as personal reasons. Work inspired by his animal model continues; this involves the development of a chronic relapsing form of experimental allergic encephalomyelitis which in important ways mimics MS.

Whether this would have remained a promising course of research, particularly without Wisniewski, is now a matter for speculation. But his departure brought more administrative problems, for he had been difficult to find. In an effort to keep the unit going the MRC cast round again for another director. One man who was invited declined, in spite of what the MRC calls "considerable material inducements". Several other candidates applied, including at least one from the unit itself. None fitted the bill. Advertisements which broadened the scope of the job to include dementias had not helped.

In May the MRC took a decision in principle to run the unit down. The Council met last week to consider an appeal from the staff, but the outcome was almost a foregone conclusion. The statement confirming that the decision would stand expressed great regret. A formal statement giving detailed reasons is expected this week. Whether this will try to explain how and why no director could be found to take the job is unclear. But in the meantime the MRC will probably be hoping that the unit it is now running down might one day start again. □

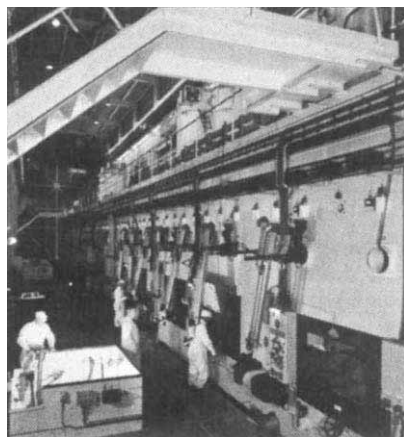
"LOST!", the *Sun's* front page headline boldly proclaimed. "Enough plutonium to make fourteen A-bombs. Finder please return to Tony Benn". It was one way—a popular daily's way—of reporting what was perhaps last week's non-story in Britain. Most other papers recognised it for what it was and gave it less prominence. But they still faced the problem of explaining that there was no problem.

The occasion was the publication by the UK Atomic Energy Authority (UKAEA) and British Nuclear Fuels Ltd (BNFL) of data on nuclear materials accounting in Britain. The figures seemed to show that, since 1970, 96.8 kg of plutonium had gone "missing" from Windscale and 97.6 kg of highly enriched uranium from Dounreay. These are the sums of annual inventory differences—of arithmetical differences between the physical inventory and the book inventory of nuclear materials coming into, moving through and leaving any particular site or part of a site.

The annual inventory difference is known as MUF—material unaccounted for. And it is never zero. In these nuclear-fuel fearing days, the idea that plutonium or enriched uranium cannot be accounted for is, well, explosive. After all, if material always seems to be missing, how is

anyone to know when it really is missing? But it does not always seem to be missing; sometimes it seems to be growing. And for the same reasons

Material aid



Decanning Magnox fuel (BNFL)

BACKGROUND

that the UKAEA and BNFL are sure that no one has given them material illicitly, they are sure no one has taken it either. Those reasons are to be found in the security measures they adopt, of which MUF is simply not an integral part.

So what is MUF? Essentially it is a

recognition of the need to account for valuable materials—a need admittedly highlighted by the first reports three months ago that a shipload of uranium disappeared in 1968 between Antwerp and Genoa and by demands from such groups as Friends of the Earth for details of nuclear materials accounting in Britain and elsewhere. But accounting has always been there; and the techniques are apparently improving. What is new are the figures themselves.

In Britain the accounting balances are struck on a quarterly or monthly basis. Their minimal value as an aid against diversion might improve if, as is said to happen at some US plants, a balance is struck once a shift. But the MUF concept was not designed for this purpose.

The figures will move closer to zero only as built-in sources of error are reduced. These include inaccuracies in, for example, estimating the amounts of plutonium in irradiated fuel elements. Estimates of plutonium at other stages are also fraught with difficulty. No one is expecting the figures to reach zero.

MUF figures justify few fears or hopes. But if the accounting wasn't there, it would have to be invented; and most people would rather have the figures calculated.

IN BRIEF

R & D statistics

Total expenditure on R & D at colleges and universities in the United States increased by 9% last year, about 2% above the rate of inflation, according to a series of figures released last week by the National Science Foundation. Within the total, however, spending on basic research rose by only 5%, less than the rate of inflation, while support for applied research rose faster than inflation.

The overall proportion of R&D funds in universities and colleges devoted to basic research has declined from 77% in 1970, to about 68% this year, the figures suggest. Earlier this year, a study of the health of academic science indicated that declining support for basic research has seriously eroded the quality of research in some institutions.

MAFF's chief scientist

After five years as Chief Scientist at the UK Ministry of Agriculture, Fisheries and Food, Sir Charles Pereira will be retiring at the end of November. Professor B. G. F. Weitz, Director of the National Institute for Research in Dairying at the University of Reading, will succeed him. Professor Weitz will inherit responsibility for identifying R & D needs and for the department as a 'customer' under the customer/contractor arrangements for applied research. He will also have £42 million at his disposal for R&D in 1977-78.

Professor Weitz's research interests have included the development of Brucella vaccines, the action of tetanus and diphtheria antitoxins and the immunological aspects of tropical diseases transmitted by insect vectors.

CERN's saving

Figures which emerged at the June session of the CERN Council in Geneva confirmed a SF50.1 million saving (at 1970 prices) on the super proton synchrotron (SPS) programme that will end in 1979. The SPS programme is now estimated at SF1,099.9 million. The Council also agreed to prolong until August 1980 the agreement CERN has with the European Southern Observatory; the 1978 budget for this is estimated at SF618.7 million.

From the beginning of next year the new leader of CERN's experimental physics division will be Dr Erwin Gabathuler (43), from Britain. He has been project leader, European muon collaboration, since 1974. Before that he was responsible for the Daresbury-based programme at CERN.

ACRONYMS are a modern nuisance, but all of us make use of them to some extent. COSPAR means the Committee on Space Research of the International Council of Scientific Unions, and this mouthful refers to an international scientific organisation concerned with fundamental research carried out with the use of rocket-propelled vehicles. COSPAR functions as a sort of scientific mini-UN, and holds annual meetings in various member countries. This year, after considerable behind-the-scenes give-and-take with the Soviets, the invitation of Israel as host had finally been accepted. There were symposia on instruments for space astronomy, on travelling interplanetary phenomena, on Viking experiments and on global food information systems. There were also, as usual, meetings of various 'working groups', all of which are recorded in the Book of Chronicles of the Goings-on in Israel (also known as the abstracts and proceedings of the twentieth COSPAR meeting).

I have been regaled many times with accounts of visits to the Holy Land, starting with Mark Twain's *Innocents Abroad*, but I found that to see Israel for the first time is a deeply cultural and emotional experience. The land stands once more at the pinnacle of a climactic episode of her history. Who can deny that the rest of the world owes something to the Jews, the Israelis, in the light of what has happened in the past 50 years? But how can I phrase these ideas adequately? I am subdued before the oratory of the great, spanning the millennia, from Elijah to Abba Eban, and by the words of the

Irishman who reminded us that the tables of the Law were graven in the language of the outlaw.

I must urge you to visit the museum of Yad Vashem in Jerusalem. We approached it through a small avenue of trees, each of which is

COSPAR in Israel**THOMAS H. JUKES**

dedicated to a Gentile who helped the Jews during the era of the Nazi holocaust. After the fire comes a still, small voice. Replicas of the Yad Vashem museum should be built throughout the world, especially in the Soviet Union, so that all may see its contents, which vividly depict what happened in Germany and in the countries occupied by Hitler, 1939-1945.

The Dead Sea is a grand object-lesson in comparative toxicity; useful

in discussions of the subject with consumers who are nervous about poisons in their food. Drinking moderate quantities of Dead Sea water is fatal, because of its high content of magnesium (14%), sodium (7%), and potassium (4%) chlorides. A drop splashed in the eye causes intense pain. Yet these salts, in lower concentrations, are essential to life. The most crucial activity of all cells, protein synthesis, depends on ribosomes, and these need magnesium ions to maintain their integrity and function. Neither can we exist without sodium, potassium and chloride ions.

The contrasts in Israel are startling. We drove, on a modern highway, out of a brand-new town with sprinklers watering the flowering oleander bushes. Soon we were in barren, rolling country where Bedouin Arabs herded goats beside tents whose design had not changed for centuries, camels who sniffed the evening, and donkeys who serenaded it under high-tension power lines. Back to the coast we rolled through the ancient city of Jaffa, and to the new University of Tel Aviv for an outdoor evening reception. Here a professor introduced the dean, in a short speech that included a gentle reminder of budgetary needs for his department. The dean, after the manner of his kind, welcomed us warmly, but made no promises about the budget. We drank the wine, labelled 'Product of Israel, Cabernet Sauvignon'. It was just like California, where most red wines seem to be labelled 'Cabernet Sauvignon'. Vive la France, vive le COSPAR!

correspondence

Torture

SIR,—In June, Amnesty International published a monograph containing the results of three research studies conducted by its Danish Medical Group¹. The studies concerned modern methods of torture. This group, which came into being in October 1974, is the first of its kind, and its pioneer work will serve as a pattern for similar groups in other countries.

One of the reasons that this group was formed was the disclosure that in certain countries doctors have co-operated in inventing new, sophisticated ways of torture that can be used without leaving any visible evidence. To be able to prove that such methods have been used, medical experts are needed. Ingenuity in this field seems to be unlimited, but with every new revelation of torture methods, one can only hope that public opinion will have a preventative or at least restraining effect on those using them.

Thanks to the initiative of the Danish doctors, Amnesty International will form a 'Medical Advisory Board' which will encourage the forming of research and action groups in other countries and coordinate their work. Such groups have come into existence in the USA, Sweden, Holland and within the near future there may be a similar group in Britain.

Forming research and action groups is one way of helping to implement the 1975 declaration against torture that was accepted in Tokyo by the WMA General Assembly. In this declaration doctors are admonished to abstain from participating in any physical or psychological torture of prisoners. The 'Declaration of Tokyo' is an important document in a time when torture unfortunately is a matter of common occurrence in a number of countries in different parts of the world.

A doctor must refuse to participate in torturing a prisoner, but should he not also actively oppose torture? The Amnesty groups of doctors are of that opinion. As an example of what official medical organisations can do, the Swedish Medical Association has faced the question of acting on behalf of individuals or minorities that have been subjected to political persecution, torture and other inhuman treatment. As a rule, Amnesty International has initiated these actions, but the asso-

ciation has also been approached independently by its members.

Naturally, the possibilities for a medical association to have any real influence are very limited. In cases where the documentation is good, however, it has been our experience that protests to respective heads of state or other appropriate authorities can benefit prisoners and give positive results.

ARNT K. MEYER-LIE

Karlstad, Sweden

¹Evidence of Torture: Studies by the Amnesty International Danish Medical Group. Pp. 40. (Amnesty International Publications: London). Originally published in Danish in *Ugeskrift for Læger*, 2 May 1977.

A combination derivation of the proton-electron mass ratio

SIR,—Atkin¹, Bastin² and Kilmister³ have recently shown that a discrete algebraic theory readily generates the structures and cardinalities of the kind contemplated by Eddington⁴. In particular, Kilmister has pointed out that algebraic structures having 10 and 136 components are inevitably perpetuated by such a combinatorial approach.

It is well known that Eddington attempted to use these two numbers to account for the proton-electron mass ratio. He proposed that this ratio be identified with the ratio of the roots of the quadratic equation.

$$10m^2 - 136m + 1 = 0 \quad (1)$$

This quadratic root ratio is 1847.6 and was an adequate fit to the known experimental data for determining the proton-electron mass ratio in 1933, when this proposal was first made. When it became clear, however, that the experimental ratio was close to 1836, Eddington, with rather intricate physical justification, added factors to his original equation to approximate the new result. This procedure has rightly been criticised. As Kilmister puts it: "This is almost certainly the wrong way to go about things. We ought to realise that if we have any tenable theory at all on this point, we have one that agrees approximately with the results. In order to obtain better agreement, we need to recast the whole theory, not to patch it up by multiplications by constants whose values differ very little from unity".

Actually, Eddington could have recast his theory by dispensing with his notorious quadratic altogether. He had to do something physically plausible

with the key cardinal numbers 10 and 136 to derive 1836. From a combinatorial point of view (with one eye open to the physics) the most straightforward procedure is the following:

if we have 136 things to choose from and need to choose 2 at a time (one for the proton, one for the electron presumably) we will have $(136)(135) = 18360$ ways of choosing. If we then have to distribute these ways of choosing into 10 parts (for the 10 gravitational potentials perhaps) we end up with the desired number

$$P_2^{136}/10 = 1836. \quad (2)$$

This is close enough to the observed ratio of 1836.109 to suggest something stronger than mere coincidence.

SAUL-PAUL SIRAG

Institute for the Study of Consciousness
California, USA.

¹ Atkin R. H. & Bastin Ted *Int. J. theor. Phys.* 3, 449-466 (1970).

² Bastin E. W. (Ted) in *Quantum Theory and Beyond* (edit. by Bastin T.) 213-226 (Cambridge University Press London 1971).

³ Kilmister C. W. *Sir Arthur Eddington* 269-271 (Pergamon Press Oxford 1966).

⁴ Eddington Sir A.S. *Relativity Theory of Protons and Electrons* (Cambridge University Press London 1936).

Proposed terminology

SIR,—George S. Kell (23 June, page 665), is to be congratulated and applauded for inviting discussion of his proposed new terminology rather than incorporating it willy-nilly in a scientific paper. If other scientists had been as modest and careful then I doubt if the jargon would have been saddled with misleading terms like charm and colour. I, for one, am not charmed by the strangeness of the particle physicist's use of colour but I am sure that many viewers of a recent BBC science programme actually believe that quarks come in pretty shades of red and green. They have colour have they not? Physicists who indulge in purloining common adjectives to describe properties which bear no relation to the original meaning of the words should pause, reflect and be glad that at some time past they had not labelled some property 'gay', as in gay particle physicist.

George Kell has made a good try at avoiding such whimsy but should glance at a dictionary of geology where he will find 'psammitic' already in use, thereby pre-empting the root of his proposed terminology.

WILLIAM WELSH

University of Aberdeen, Scotland

news and views

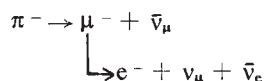
The Twin Paradox revisited

from Tom Wilkie

THE muon may be a curious particle, but the study of its properties is a fruitful one (see *Nature* **267**, 582; 1977). The accurate determination of the muon lifetime, reported in this issue (page 301), has also provided a stringent check on Einstein's Theory of Special Relativity. As an added bonus, it sheds light on the 'Twin Paradox', gives an upper limit to the granularity of space-time, and tests CPT invariance of the weak interaction. The precision of the experiment is to within 0.1%, presently the most accurate test of Special Relativity using elementary particles. It was carried out using the CERN Muon Storage Ring which was built to measure the anomalous magnetic moment of the muon and so test Quantum Electrodynamics (QED). The present experiment is doubly welcome, in that it continues the desirable trend for elementary particle experiments of very high precision, and in that it makes good use of already existing apparatus. Too often in the past, particle physicists have been content only with gross trends in their data; too often also, there have been demands for very expensive equipment to perform a single experiment. In the autumn the Ring will be adapted to accelerator physics, and will start a new life investigating the exciting field of stochastic and electron 'cooling' of proton beams (*Particle Accelerators* **7**(4); 1976).

As a species of massive electron, the muon is a charged particle with intrinsic spin $\frac{1}{2}\hbar$; it therefore has a magnetic moment when placed in an external magnetic field. It is unstable; in the laboratory it decays into an electron and two neutrinos, in approximately 2.2 μ s. Recently, however, there has been intense theoretical speculation that, within the context of non-abelian gauge theories, the muon may decay directly into an electron and a photon (thus violating muon conservation number). These suggestions (*Phys. Lett.* **67B**, 303 & 309; 1977) have not yet been verified experimentally. The conventional muon decay pattern has a

characteristic 'sickle' shape corresponding to



This distinctive pattern is seen when the pions are stopped in a bubble-chamber, and allowed to decay at rest. In the CERN experiments, however, the pions are injected into a 14-m diameter storage ring, and allowed to decay in flight. Because this decay violates parity, the produced muons are spin-polarised and their observed decay products, the electrons, are emitted along the direction of the muon spin. It is this ability to measure the muon spin direction which allows an accurate value of the anomalous magnetic moment to be found. The theory of QED, which essentially marries Quantum Mechanics and Special Relativity, predicts certain corrections to the naive, non-realistic value of the magnetic moment. The beautiful experiment done at CERN gave a triumphant measure of these corrections (Bailey *Phys. Lett.* **68B**, 191; 1977)

$$a_\mu (\text{expt.}) = (1,165,922 \pm 9) \times 10^{-9}$$

$$a_\mu (\text{theory}) = (1,165,921 \pm 10) \times 10^{-9}$$

Although this result may be considered a very complete confirmation of QED as the most accurate physical theory ever devised, it is at best an indirect test of Special Relativity. Because the muon is unstable it can be regarded as a subnuclear 'clock', and used to measure the time dilation effect predicted by Einstein. If two identical clocks are in uniform relative motion and an observer, considering his clock as stationary, compares the time measured by the two clocks, he will see the 'moving' clock losing time as it recedes from him. However, an observer with the 'moving' clock will consider himself to be stationary, and observe the first one to be going slow. This time dilation has aroused some passion (*Nature* **255**, 519; 1975). The so-called 'Twin' or 'Clock Paradox' arises when the 'moving' clock is later returned to the stationary one; it is

indeed seen to have measured a smaller time-interval. But why could we not have regarded the 'moving' clock as stationary, and expected the first observer to have aged less? The resolution is that the 'moving' system has to change its velocity (that is, undergo acceleration) to return to the stationary one; the two are not equivalent, inertial frames in the sense of Special Relativity.

The existence of cosmic-ray muons at ground level supports the idea of time dilation; for, if the muons' lifetime is not lengthened during flight, they would all decay in the upper atmosphere. There have been more precise experiments using the line-of-flight decay of accelerator-produced pions and kaons (*Nucl. Phys.* **48B**, 343; 1972; *Phys. Rev. Lett.* **34**, 1244; 1975). The advent of high-precision caesium atomic clocks has made the macroscopic test of time dilation possible. Hafele and Keating (*Science* **177**, 166; 1972) simply loaded such a clock on to a commercial jet airliner on a round-the-world trip! Although reporting a positive effect, the experimental errors were substantial.

A different approach (*Phys. Rev. Lett.* **4**, 165; 1960) uses the Mössbauer effect to examine the 'Twin Paradox'. Photons from ^{57}Fe decay at the centre of a rotor enter a resonant absorber on the perimeter, and so are prevented from registering in an external, stationary counter. When the rotor is spun rapidly, the count rate goes up as the moving absorber is no longer in resonance with the stationary emitter—its time has been dilated.

Similarly, the muons circling in the CERN storage ring at 99.94% of the speed of light closely fulfil the role of moving clocks. To move in a circle they must undergo a centripetal acceleration, that is, they are in a non-inertial frame, and so they mimic the moving twin. We would expect the CERN muons to remain younger than their stay-at-home brethren. It is indeed observed that the moving muons live longer in agreement, to one part in 10^3 , with the predictions of Special Relativity. The stationary twin's time-scale is given by observation of

muonium decay (*Sov. Phys. JETP* **40**, 811; 1974).

One of our basic theoretical assumptions is that of CPT invariance: the physical properties of particles and anti-particles should be identical, apart from their charge. It is reassuring that the CERN group should have accurately verified this also.

But one of the most interesting consequences of this experiment straddles both Special and General Relativity. Any test of Special Relativity is, ultimately,

a test of the homogeneity of space-time. Is it possible that space itself is quantised; that there is a fundamental distance below which we cannot probe? There has been some theoretical speculation that the existence of miniature black holes would entail such a fundamental distance. The CERN experiment puts an unambiguous upper limit ($\sim 10^{-13}$ cm) on the granularity of space-time. In so doing, it places a lower limit on the mass of any black hole. $\square$

the clearance of forests and their replacement by agricultural systems which must be considered carefully. First, there is the extent of forest cover on our planet; Bolin suggests that dense forest covers about 30% of the Earth's land surface and contains about 90% of the Earth's living biomass. Obviously, clearance of forest results in an initial movement of carbon from a living reservoir to the atmosphere, since most forest clearance is accompanied by the burning of waste timber, and most wood used for construction or pulp will be converted into CO_2 after a lag phase (which will be very short in the case of pulp).

Although the forests represent a major carbon reservoir, it would be wrong to consider them all as an efficient carbon sink. Most natural forest vegetation is at or near a climax or equilibrium state. Biomass is at or close to the maximum attainable within the existing constraints of climate, which means that the gross production of the system will be matched by the combined respiration of autotrophs and heterotrophs (herbivores, carnivores, detritivores and decomposers) within the system (see Odum *Science* **164**, 262; 1969). Thus the climax forest is in a no-growth situation and its carbon uptake will be equalled by its carbon loss. Even fairly mature forests which have not yet attained climax conditions may be past their peak in terms of net ecosystem production.

Thus, to act as an efficient sink for atmospheric carbon, an ecosystem should ideally have a high net ecosystem production rate. The net primary production rate is not necessarily a good index of net ecosystem production. According to Odum net ecosystem production (biomass growth rate) reaches a peak in mid stages of succession.

If one were to consider forest clearance purely from the point of view of global carbon balance (which is, of course, equivalent to wearing ecological blinkers), it represents the creation rather than the destruction of a carbon sink in that it revitalises successional processes. It raises atmospheric carbon, however, by converting biomass carbon to CO_2 and by accelerating the decomposition of soil organic matter and plant litter.

The question must be raised whether one could use a fast-growing terrestrial biomass to compensate for the industrial injection of fossil carbon into the atmosphere. Some of the possibilities in this sphere have recently been considered by Dyson (*Energy* **2**, 287; 1977). Dyson concentrates upon the use of fast growing tree species and also wetland plant species as possible implements for atmospheric CO_2 control. For

Atmospheric carbon dioxide and forest clearance

from Peter D. Moore

THE build up of carbon dioxide in the atmosphere is now firmly established, although its consequences are still a matter for debate. The frequently quoted figures from Mauna Loa in Hawaii demonstrate a rise in atmospheric CO_2 from levels of 313 p.p.m. in 1958 to 327 p.p.m. in 1975. It is generally accepted that the bulk of this rise is due to the injection of CO_2 into the atmosphere as a consequence of the burning of fossil fuels. It is also likely that the oxidation of carbon contained in living (mainly plant) biomass and in soil organic matter and litter has contributed to the rise. Farmer and Baxter (*Nature* **247**, 273; 1974) for example, have published data which implies a considerable increase in atmospheric CO_2 , perhaps as much as 20 p.p.m., during the first two decades of this century, and they considered that this could be accounted for by the oxidation of non-fossil organic carbon.

On the other hand, one might expect the raised atmospheric levels of CO_2 to result in a higher gross production on the part of photosynthetic plants. CO_2 is the limiting factor for rates of photosynthesis and raised CO_2 levels are widely used to enhance the growth of greenhouse crops. It is not unreasonable to hope, therefore, that global carbon fixation is increasing as a function of CO_2 concentration. Such an hypothesis is difficult to test, however. Hall, Ekdahl and Wartenberg (*Nature* **255**, 136; 1975) attempted an analysis of the Mauna Loa data which was designed to isolate CO_2 fluctuations due to changes in the biospheric reservoir of carbon. They estimated the month-by-month variation in CO_2 output by industry in the Northern Hemisphere and used the curves produced to correct the ambient CO_2 record over the period 1958–1972.

Making allowance for oceanic uptake (estimated at 42%) they could then obtain values for the overall photosynthetic and respiration of the biosphere by comparing the differences between the succeeding seasonal peaks and troughs in the atmospheric CO_2 concentration. Their conclusion was that there had been no basic change in the pattern of biosphere metabolism. An alternative way of interpreting their data would be to postulate that any increase in production has been matched by an increase in respiration, or its chemical equivalent, biomass oxidation by combustion.

Bolin (*Science* **196**, 613; 1977) has recently discussed the influence of changes in land biota on the carbon cycle and he also emphasises the influence of forest clearance and agricultural development on the atmospheric carbon load. According to his calculations the atmosphere contains about 690×10^9 tons of carbon (327 p.p.m.). Over the last decade the input from fossil fuel combustion has averaged 4.0×10^9 ton yr^{-1} , but at the same time there have been changes in the biosphere which will also have led to a net input of carbon to the atmosphere. Bolin estimates these rates at: deforestation and fuel wood production, 1.1×10^9 ton yr^{-1} ; changes in organic matter in the soil, 0.3×10^9 ; less the drain on the atmospheric carbon level resulting from reforestation, estimated at 0.3×10^9 . Thus the overall net injection of carbon into the atmosphere due to human activity averages about 5.1×10^9 ton yr^{-1} .

Observations on atmospheric carbon suggest that only about 40% of this remains airborne, the rest being absorbed by the oceans plus (in Bolin's opinion) a small loss of carbon from the atmosphere created by the regrowth of vegetation following clearance.

There are several points concerning

example, the American sycamore (*Platanus occidentalis*) has a production capacity equivalent to 750 tons of carbon $\text{km}^{-2} \text{yr}^{-1}$. At this rate one would need $7 \times 10^6 \text{ km}^2$ to be planted with the tree in order to compensate fully for rising global atmospheric CO_2 . Dyson claims that the United States could provide $7 \times 10^5 \text{ km}^2$ of suitable land, but he fails to take into account the loss in CO_2 consumption resulting from replacement of low grade forest currently occupying such areas. Even more important is what you do with the crop once it reaches maturity. The essence of atmospheric CO_2 control is to lock up the carbon in an alternative, permanent reservoir. Sadly, organic carbon is only too readily oxidised and returned to the atmosphere as CO_2 . It could be retained as a forest biomass, but pressure on land is unlikely to permit this.

A natural ecosystem, with an additional built-in carbon reservoir is the peat-forming system. But the bulk of the world's peatlands occur in high latitudes, are generally low in nutrients and have low productivity. Interna-

tional Biological Program data collated in *Primary Production and Production Processes, Tundra Biome* (edited by Bliss & Wielgolaski, Dublin, 1973) show that most tundra mires have annual productivities of only about 40–100 g m^{-2} . The raised mires of Britain have average peat accumulation rates of only about 4–10 cm per century (Hibbert & Switsur *New Phytol.* 77, 793; 1976). Thus these systems cannot be regarded as very efficient carbon sinks. The more productive wetlands, such as *Typha* and *Phragmites* swamps and ecosystems dominated by the tropical water hyacinth (*Eichhornia crassipes*), present problems of carbon storage and the inhibition of decomposition.

One of the most critical limitations on any such use of biomass growth as a carbon sink is the energy expenditure necessary for the establishment, management, fertilisation, harvest and storage of the crop. Where energy consumption is still closely linked with CO_2 production one wonders whether any attempts at biological control of CO_2 will prove self-defeating. □

New quarks . . . now the good news

from D. Morgan

A conference on Particle Physics was held at Budapest on July 4–9, 1977.

THIS year's high energy physics conference held at Budapest provided a rich harvest of new experimental results powerfully reinforcing present trends in the subject. Most challenging was the report by Leon Lederman (Columbia University) of a new vector meson or group of vector mesons at 9.5 GeV which could very well signify a new form of hadronic matter. Great interest also attached to the first results of neutrino experiments on the new 400 GeV European SPS accelerator at CERN, Geneva. Both these and many other of the results reported at the conference bear on an outstanding theme of contemporary particle research—the structure of matter as seen through the weak and electromagnetic currents.

Present thinking is here dominated by a fusion of two sets of ideas—the unified gauge theories of weak and electromagnetic (current) interactions, and the quark parton model which extends the description of current inter-

actions to the world of strongly interacting particles. The outcome is a very close analogy between the non-strongly interacting 'leptons' (the electron, the muon and their associated neutrinos) and the quarks from which the strongly interacting 'hadrons' are built. Before 1974, three types of quark sufficed to build the known hadrons. One of the great successes of the unified model was to predict the existence of a fourth type of quark, a prediction which was triumphantly vindicated by the discovery of the J/ψ family of 'new' particles. An implication of this whole way of thinking is that the best way of studying new types of hadron is through their weak and electromagnetic interactions. Hence the tremendous contemporary emphasis on neutrino experiments, on the study of e^+e^- annihilation and on the observation in hadron collisions of systems decaying to leptons. Objectives are to discover what are the constituents of matter, both lepton and quark, and how they couple to the weak and electromagnetic interactions: which of the alternative gauge theories is that chosen by nature. In a world with just four sorts of quark and four sorts of lepton, a simple possibility is afforded by the $\text{SU}(2) \times \text{U}(1)$ model of Steven Weinberg and Abdus Salam which success-

fully predicted the existence of neutral weak currents and which still gives a good description of neutrino phenomena, according to new data presented to the conference. There are nonetheless good reasons for exploring wider possibilities. In the first place, there exists increasingly secure evidence for a new heavy lepton τ of mass 1.8 GeV, which presumably has a companion neutrino ν_τ . The unified models favour schemes with an equal number of quarks and leptons, suggesting the existence of two more new kinds of presumably rather heavy quarks, nicknamed t ='top' and b ='bottom', and associated effects in the hadron spectrum. The current couplings of the extra quarks and leptons could simply replicate those of their lighter brothers or could take new forms. The place to seek such effects should be in neutrino experiments and in experiments probing the coupling to lepton pairs. Great interest had therefore attached to reports from a counter experiment at Fermilab of an anomalously large production of high mass hadron systems in high energy anti-neutrino reactions (the so-called 'high-anomaly'). This suggested not only the coupling of a new kind of quark (the bottom quark has suitable properties) but a new kind of coupling involving the opposite (right-handed) helicity states from those implicated previously. It is against this background that the new experiments are to be judged.

Neutrino experiments

The outcome of the new high-statistics CERN experiment reported by Jack Steinberger is completely to demolish the high- y anomaly in anti-neutrino reactions. Both for neutrinos and anti-neutrinos the new inclusive cross-sections very much reinforce trends previously perceived in experiments at lower energies and in deep inelastic charged lepton scattering. For proponents of new quarks this is the bad news.

New vector meson

The discovery of the new 9.5 GeV vector meson very much mirrors that of the J/ψ (3.1). Like the 3.1, the new 9.5 GeV effect is seen in the $\mu^+\mu^-$ spectrum from very high energy proton-nucleus collisions. The new data comes from an experiment designed with extreme care to maximise the sensitivity to small signals. This results in an extremely clean unambiguous signal for one or more resonances spanning a range of 1.5 GeV centred at 9.5 GeV, the signal being seen against a background which falls smoothly from 4 to 14 GeV. The resolution is insufficient to reveal narrow

structures so a full analogy with the J/ψ and its associated spectrum is not established. Nonetheless, it seems extremely likely that we are getting a first glimpse of a further 'new' spectroscopy involving one or more new quarks—an exciting prospect for the new e^+e^- storage rings at Cornell, Hamberg, Novosibirsk and Stanford which will be capable of studying the new effects with much better resolution.

Many other new results and problems were discussed at the conference. From Stanford came news of a new recruit to the J/ψ spectrum of mass 3.77 GeV which provides rather striking confirmation of the accepted picture of these states. (It is of course upon this picture that the optimistic hopes for a further new spectroscopy depend.) There was also progress in the field of 'old' mesons with indications that the long awaited A_1 axial vector meson may at last have been established; also reported signals for new vector mesons in the 1–2 GeV range. A further intriguing contribution concerned very narrow effects seen in pp annihilation. There continues to be much speculation as to the structure of the weak interactions and of the lepton spectrum. With couplings as in the Weinberg–Salam model, one predicts specific parity violating effects in atomic spectra, effects which have been

sought and not found in recent experiments at Oxford and Seattle. The consequent urge to examine alternative coupling schemes was for a time reinforced by unconfirmed reports of an appreciable $\mu \rightarrow e\gamma$ decay rate. It is natural to associate such lepton number non-conserving processes with non-vanishing neutrino masses. The consequent possibility of neutrino mixing was discussed at the conference by Bruno Pontecorvo. A further unresolved question in the field of leptons is posed by the observation of trimuon events of which a small number, mostly involving very large muon momenta have been observed by the same Fermilab group which saw the former high- y anomaly. Despite the current preoccupation with leptonic probes, the quark structure of hadrons is also being explored in wide angle hadron–hadron collisions notably at the CERN ISR from which came further information on the formation of hadronic jets. The outstanding problem for theorists is to understand the detailed mechanism for confining quarks in hadrons. Several interesting lines of work were reviewed but we still await solutions. A high spot of the conference was an evening lecture by Paul Dirac describing his epoch making discoveries of 50 years ago—an object lesson in how to find solutions, and in humility. $\square$

Oxford University). It has been demonstrated that the quantum efficiency for the photochemical generation process is readily determined from the angular velocity dependence of the current, and the technique is now being applied to investigate the mechanism and efficiency of photogalvanic cells based on dissolved, absorbing species.

Electrochemical photogalvanic cells represent a particularly attractive technology for solar power generation, since in principle they are free of some of the problems associated with solid state solar cells. There is no need to match the physical properties of the materials forming the junction in a solution cell and polycrystalline semiconductor electrodes have already yielded power efficiencies greater than 5%. However, liquid solar cells have their own unique problems, one of which is photocorrosion of the semiconductor. Rotating ring-disk and sinusoidally modulated rotating disk electrodes were used (B. Miller, Bell Laboratories, Murray Hill, New Jersey) to study polycrystalline cadmium sulphide semiconducting electrodes in $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ and $\text{S}^{2-}/\text{S}_x^{2-}$ media. Details were given on the means for establishing the potential at which significant photocorrosion begins. The $\text{S}^{2-}/\text{S}_x^{2-}$ system was found to be far less susceptible to photocorrosion.

The use of electrochemistry as a tool to study fluid dynamics is becoming more common. Non-newtonian fluid studies at rotating disk electrodes have led to the verification of an inversion of flow direction at a critical rotation speed. Also drag reduction in turbulent flow in the presence of poly(ethylene oxide) has proved especially amenable to study with the rotating disk and rotating ring electrodes (L. Viet, CNRS, Paris).

A rotating disk study of the chemical vaporisation of silicon (M. L. Hitchman, RCA, Zurich) from SiCl_4 and H_2 permitted the separation of transport and chemical processes, and the determination of the heat of activation for the silicon deposition process. This approach appears to hold considerable promise for the study of analogous gas–solid reactions.

G. K. Batchelor presented Levich's plenary paper. Levich, who requested permission to emigrate from the Soviet Union several years ago, was not allowed to attend the conference. This paper was a comprehensive review of the entire field and delineated problem areas. In the discussion of heterogeneous chemical kinetics, the necessity for uniform accessibility of a surface, or calculable mass transport rates, was stressed. The paradox of the existence of a rigorous statistical theory and several semi-empirical theories of

Physicochemical hydrodynamics

from Stanley Bruckenstein

The Levich Birthday Conference was held in Oxford on 11–13 July, 1977.

THE Conference, organised to honour Benjamin G. Levich's 60th birthday, aimed at assessing the present state of development of physicochemical hydrodynamics and its future thrusts. Physicochemical hydrodynamics was the name coined by Levich in 1952 to describe problems related to the influence of fluid flow on chemical or physical transformations, as well as the influence of physicochemical factors on fluid flow. The ideas in Levich's 1952 book and the Soviet work underlying it, were for the most part, overlooked until 1962 when his monograph was translated from the Russian into English. Almost immediately, research

in certain aspects of electrochemistry and fluid mechanics began to reflect the influence of the Soviet literature and the Oxford Conference is a measure of western progress and innovation made in the past 15 years in this interdisciplinary field. About 70 papers were presented in two parallel sessions, which were attended by more than 100 chemists, engineers and physicists. The conference concerned itself with six topics: physical transport, interface mechanics, interspersed phases, chemical hydrodynamics, electrochemistry and physics.

The use of controlled mass transport in experimental electrochemistry has made a tremendous impact. The principal convective–diffusion controlled electrode systems in use today are based on the rotating disk and rotating ring-disk geometries. Semitransparent disks are being used to detect electrode intermediates produced photochemically in the diffusion layer adjacent to the electrode surface (W. J. Albery,

turbulence was discussed. Levich remarked that, despite their obvious weaknesses, the semi-empirical approaches did permit successful calculations in very complex flow situations. There is need for a more detailed study of the structure of turbulence at rough walls. Levich believes that the phenomena of higher viscosity at the interface of two fluids is due to the presence of surface active agents, not a "surface viscosity". The mechanism of the breaking of bubbles in liquid media, ascribed to internal motions of the gas in the bubble is not yet confirmed by convincing experimental data. In his discussion of statistical systems (a stochastic and disorderly flow regime in space and time) a number of unsolved problems were discussed, including that of rising bubbles in a liquid, breaking of a gas stream through a liquid layer, and the process of heat and mass transfer in a system of bubbles. □

Isotherm limits ocean magnetism

from Peter J. Smith

THE linear marine magnetic anomalies which have provided such important evidence for seafloor spreading must result from magnetised material within the crust and, possibly, the uppermost mantle. To say that, however, is to state little more than the obvious, although to go beyond it is difficult. With insufficient direct information from, say, deep drilling into the ocean floor it is impossible to constrain the variables in theoretical models of the magnetised zone in such a way as to provide a unique solution, even when the model is made unrealistically simple. For example, the deceptive regularity of the anomalies themselves gave rise in the first instance to an overly simplified model comprising alternating blocks of normally and reversely magnetised rock; but the magnetisations and vertical thicknesses of the blocks could be varied independently within certain limits to permit a large number of viable block arrangements.

In early versions of the alternating block model, block thicknesses ranged up to at least 9 km. Later, however, Carmichael (*Can. J. Earth Sci.* 7, 239; 1970) and others concluded that if the high magnetisations found in rock

samples dredged from the ocean floor were typical of those throughout the blocks, the magnetic layer could be as thin as 0.4–1.0 km. Indeed, this view proved so popular that the most common block thickness used in anomaly modelling ever since has apparently been 0.5 km (with magnetisations in the range 8×10^{-3} – 20×10^{-3} emu cm⁻³). But as Kristjansson and Watkins (*Earth planet. Sci. Lett.* 34, 365; 1977) now re-emphasise, no one should make the mistake of regarding this thickness as anything more than a "computational artifact". It serves a useful purpose in anomaly modelling but bears little relation to reality.

The reality is that the zone producing the observed anomalies is likely to be structurally complicated, chemically and mineralogically inhomogeneous, the product of emplacement within an active ridge zone of variable width, and affected by post-emplacement rotation. Such complexities are currently the subject of intense study, and models which attempt to accommodate them are already beginning to appear. Kristjansson and Watkins, on the other hand, have taken a more direct approach in seeking information about the actual thickness of the magnetic zone beneath the active central volcanic area of Iceland. This area is, of course, the landward continuation of the oceanic Reykjanes ridge; and although the magnetic anomalies are less sharp than those over oceanic ridges proper, they may still be used to determine a magnetic zone thickness. Thus if the zone has an average magnetisation comparable to that of the surface basalts (15×10^{-3} – 20×10^{-3} emu cm⁻³) it need only have a thickness of 0.5 km to permit modelling of the overlying anomalies. Alternatively, if the observed proportion of tuff and breccia at the surface is taken to obtain at depth, the net magnetisation is more likely to be 6×10^{-3} – 8×10^{-3} emu cm⁻³, leading to a magnetic zone thickness of about 2 km. Either way, the "computational" thickness is not very great.

But again the reality is different, at least if the magnetic properties of igneous samples from eight deep (up to 2 km) boreholes are anything to go by. The room temperature magnetic susceptibility, for example, is highly variable (depending to some extent on rock type) but shows no systematic change with depth up to the 2 km limit. And since the boreholes lie in regions of high thermal gradient, geothermal activity and recent volcanism, this is equivalent to saying that the susceptibility shows no systematic change with zones having present temperatures of up to about 300 °C. Outside the highly thermal areas, however, the 300 °C isotherm corresponds to a

depth of about 4.5 km. The implication therefore is that beneath parts of the central volcanic area of Iceland the magnetic zone extends to depths of at least 4.5 km.

This is much greater than the greatest depth deduced from magnetic anomalies, confirming that the latter is indeed a fiction. It also suggests to Kristjansson and Watkins that to think in terms of a physically discrete magnetic layer of any thickness is fundamentally wrong—a point which needs to be made if only because some workers have already shown a tendency to translate a mathematical convenience into a sort of reality by thinking aloud in terms of a distinct magnetic layer with a base accessible in principle to sampling (for example, by the Deep Sea Drilling Project). The point that Kristjansson and Watkins wish to emphasise is that in their view there is absolutely no reason to suppose that the lower limit of magnetisation, which must exist, is governed by anything other than the Curie point isotherm. Or to put it another way: there is no reason to suppose that the material immediately below the magnetised zone is any different from that in the zone itself. The former is simply at a temperature high enough to preclude its being magnetised. □

Magnesium and energy

from E. F. Emley

An international conference on Energy Conservation in Production and Utilisation of Magnesium was held at the Massachusetts Institute of Technology on 25–27 May, 1977. The conference was co-sponsored by the MIT Center for Materials Science and Engineering, the US Energy Research and Development Administration (ERDA) and the International Magnesium Association.

"In the opinion of knowledgeable materials engineers, magnesium is a structural metal whose time has come". This opening sentence from the announcement set the tone of the conference, which set out under the guidance of M. C. Flemings to "create a meaningful dialogue among industrial, university and government personnel that will lead to energy con-

Peter J. Smith is a Reader in the Department of Earth Sciences at the Open University.

E. F. Emley is Group Chief Metallurgist of the British Aluminium Company Ltd.

servation in the production of magnesium and through its utilisation".

For many years the use of magnesium has been hindered by factors such as limited sources of supply of the metal and smallness of demand—about one fiftieth of that for aluminium—which in turn have prevented realisation in production of the benefits of scale. General structural and aerospace uses have tended to decline and the growth of demand has reflected increasing use for non-structural applications, such as alloying with aluminium and desulphurising steel. Realisation that magnesium is a non-strategic material (sources, including seawater, are inexhaustible and very widely distributed) coupled with the possibilities of energy saving in applications where weight or inertial forces are important, has brought nearer the time when new investment on a scale that could lead to cost reductions can be contemplated. Meanwhile, important improvements to both the electrolytic and the thermal reduction routes were announced at the conference, and these alone should lead to substantial reductions in energy consumption and production cost.

At the first session, J. A. Barclay (US Bureau of Mines) set the scene by calculating the relative total energy consumption for producing aluminium and magnesium, and suggesting directions in which improvements might be sought. Converted to kwh(t)/tonne, his figures were 71,500 for aluminium by the Hall-Héroult route and 105,000 for magnesium by the Dow seawater electrolytic process, with savings of 9% and 6% for the ferrosilicon (Pidgeon version) and carbothermic processes respectively. Comparative figures presented by P. Lugagne (Société Française d'Electrometallurgie) indicated a net energy consumption of 76,500 kwh(t)/tonne for the Magnetherm version of the silico-thermic process, which includes allowances for the energy content of saleable by-products amounting to about 15% of the total. A similar energy credit would be available to the electrolytic process if starting from brine, by selling the by-product chlorine. Looking to the future he foresaw that in 1990 the energy requirements to produce 1 tonne of each metal would be virtually the same. In similar vein, projections by R. B. Stein (Bechtel Corporation) indicated that the price curves for virgin aluminium and magnesium could cross about 1985.

It was confirmed at the meeting that Norsk-Hydro will bring on stream next year new plant involving the dehydration of waste $MgCl_2$ brines from the German potash industry. This will give rise to 2.8 tonne chlorine per

tonne of metal produced, and lead to a substantial net energy gain. Dow have an apparently similar development in hand which is expected to result in an energy saving of 30%.

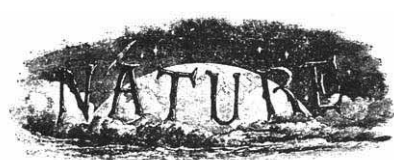
A high spot at the meeting was the announcement that J. M. Avery, well known for his contributions to blast-furnace technology, had invented a continuous version of the Magnetherm ferrosilicon reduction process, operating at atmospheric pressure, and that agreement had been reached with the Arthur D. Little organisation to evaluate the process.

Following consideration of primary production, the conference turned to energy usage in fabrication, and the comprehensive data of S. L. Couling (Battelle), showed that the most favourable fabricating method, in terms of energy requirement, is hot-chamber die-casting. To deform equal volumes of aluminium or magnesium requires roughly the same energy, but less efficient furnaces and higher melt losses increase the energy requirements for wrought magnesium, as does the practice of pre-extruding stock for the extrusion process.

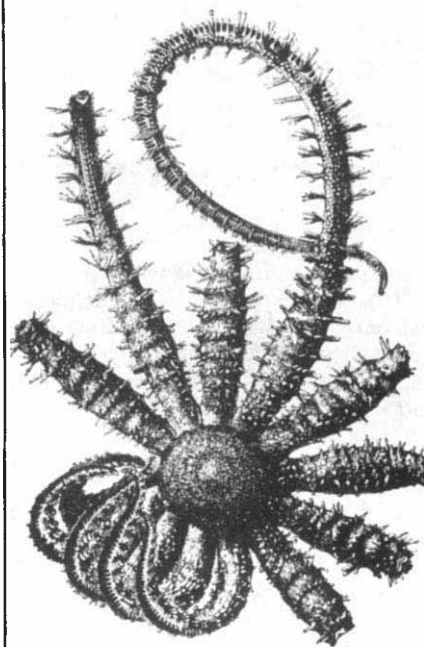
As regards growth of structural uses, M. S. Holland (Ford Motor Co.) saw the potential for magnesium in the automotive field as excellent, but not at the present price. With a primary Mg/Al price ratio of 2:1, as at present, the 1985 potential is seen as 5,000–10,000 t, whereas with a price ratio of 1.5:1 it could be as high as 100,000 t—more than the present day total US usage. Overall energy changes on replacing steel by aluminium or magnesium were further considered by J. P. Clarke and G. S. Kennedy (MIT) who found total energy requirement ratios close to 1.45:1 for each of the light metals compared with steel as unity when non-transport applications were considered. In automotive applications there can be an energy credit for the light metals owing to weight reduction and consequent fuel savings during the operational lifetime of the component, and this advantage is about 80% greater for magnesium than for aluminium.

The last day of the conference was devoted to R and D Workshops dealing with fabrication, properties, recycling, policy and, by popular request, primary extraction. Several workshops stressed the importance of price, and the need to get down to a price ratio of 1.5 compared with aluminium. Meanwhile the use of hot-chamber die-castings should be promoted, but there is need also for lower cost sheet and extrusions, for a metal-cleaning method without using flux, for a way of recovering metal from crucible residues, for ways of removing nickel, copper

and tin from scrap, for reducing magnesium losses in the aluminium industry, for new approaches to sheet such as strip-casting and the pendulum mill, for avoiding the scalping and double extrusion of billet, for better or cheaper systems of protection against corrosion, for die-casting alloys with better elevated-temperature properties and reduced tendency to surface segregation, for stronger wrought alloys—the needs are many and the challenge to the technologist, whether in industrial, academic or government service, is great. □



A hundred years ago



NEARLY a quarter of a century ago the celebrated poet and naturalist P. Chr. Asbjørnsen, was dredging in the interior of the picturesque Hardangerfjord, when, at a depth of about 200 fathoms, the dredge brought up a wonderful new star-like Echinoderm, quite unlike any form that had been up to that moment described.

In its unlikeness to most recent forms of star-fishes he saw its connection with certain fossil forms, and in its brilliant sun-like form he was reminded of the "Brising" which, according to ancient Norwegian tradition, was concealed by Loke in the abyss of the primeval ocean, but which had so long served as the ornament to cover the breast of the god Freya, and he gave the name of Brisinga to the new genus.

From *Nature* 16, 26 July, 249; 1877.

articles

Measurements of relativistic time dilatation for positive and negative muons in a circular orbit

J. Bailey

Daresbury Laboratory, Warrington, Lancashire, UK

K. Borer

Physikalisches Institut, Universität Beon, Bern, Switzerland

F. Combley

Department of Physics, University of Sheffield, Sheffield, UK

H. Drumm

European Organization for Nuclear Research, Geneva

F. Krienen

European Organization for Nuclear Research, Geneva

F. Lange

Institut für Physik der Universität Mainz, Mainz, FRG

E. Picasso

European Organization for Nuclear Research, Geneva

W. von Ruden

Institut für Physik der Universität Mainz, Mainz, FRG

F. J. M. Farley

Royal Military College of Science, Shrivenham, Wiltshire, UK

J. H. Field

European Organization for Nuclear Research, Geneva

W. Flegel

European Organization for Nuclear Research, Geneva

P. M. Hattersley

Department of Physics, University of Birmingham, Birmingham, UK

The lifetimes of both positive and negative relativistic ($\gamma = 29.33$) muons have been measured in the CERN Muon Storage Ring with the results

$$\tau^+ = 64.419 (58) \mu\text{s}, \quad \tau^- = 64.368 (29) \mu\text{s}$$

The value for positive muons is in accordance with special relativity and the measured lifetime at rest: the Einstein time dilation factor agrees with experiment with a fractional error of 2×10^{-3} at 95% confidence. Assuming special relativity, the mean proper lifetime for μ^- is found to be

$$\tau_0^- = 2.1948 (10) \mu\text{s}$$

the most accurate value reported to date. The agreement of this value with previously measured values of τ_0^+ confirms CPT invariance for the weak interaction in muon decay.

MEASUREMENT of the lifetime of a sample of radioactive material which is moving with a known velocity is a means of testing the so-called time dilation, or slowing down of moving clocks, predicted by the special theory of relativity. If, in addition the radioactive particles move in closed circular orbits then the conditions simulate those of the outward and return journey of the twin paradox in which, according to the theory, the journeying twin ages more slowly than the one who stays at home. In this situation it is also possible to search for any modification of the predictions of the theory due to acceleration.

A device which stores unstable elementary particles of high momentum is an excellent tool with which to carry out such measurements and here we report the results obtained with the Muon Storage Ring at CERN. Muons have a lifetime at rest of about 2.2 μs and decay into electrons neutrinos and anti-neutrinos

$$\mu^- \rightarrow e^- + \nu_\mu + \bar{\nu}_e$$

The decay electrons are readily detected. If the muon sample has a velocity v then the lifetime of the sample as measured in

the laboratory is given by

$$\tau = \tau_0 / [(1 - (v/c)^2)^{1/2}] = \gamma \tau_0$$

where τ_0 is the lifetime for the particle at rest.

The previous Muon Storage Ring experiment at CERN reported¹ a value of the muon lifetime in flight for a relativistic factor $\gamma \simeq 12$, which agreed within 1% with the predicted value obtained by applying the above Einstein time dilation factor² to the measured lifetime at rest³. Here we report separate measurements for μ^+ and μ^- , with a γ factor of 29.33, which are an order of magnitude more precise and which show that the predictions of special relativity obtain even under accelerations as large as 10^{18} g and down to distances less than 10^{-15} cm.

In the latest Muon Storage Ring⁴⁻⁹ muons of momentum 3.094 GeV/c ($\gamma \simeq 29.3$; $\beta = v/c = 0.9994$) circulate on orbits of 14 m diameter in a uniform magnetic field and a weak focusing electric quadrupole field¹⁰. The main purpose of the project was to measure the anomalous magnetic moment of the muon by observing the relative precession of its spin with respect to its momentum. The muons are born from pion decay

$$\pi^- \rightarrow \mu^- + \bar{\nu}_\mu$$

and by careful momentum selection in this process it is possible to obtain a circulating muon sample with high initial longitudinal polarisation. Decay electrons from the stored muons are detected in 20 shower counters inside the ring. By selecting only high energy electrons (forward going in the muon rest frame) it is possible to follow the muon spin precession since the decay electron distribution is asymmetric with respect to the muon spin direction. The decay times are measured to high precision¹¹⁻¹³ so that the muon laboratory lifetime can be obtained by fitting the observed decay electron time spectrum which consists of an exponential decay modulated by the muon spin precession frequency (ω_a).

This spectrum is distorted by small systematic effects, however—notably the loss of muons from the trapping region before decay, the dependence of the background count rate,

and the variation of the decay electron detection efficiency as a result of gain changes during the muon storage period. The resulting modification to the normal modulated exponential decay spectrum may be expressed as

$$N(t) = N_0[1 + SG(t) + F(t)]\{\exp(-t/\tau) \times [1 - A \cos(\omega_a t + \phi)] + B(t)\} \quad (1)$$

The functions $SG(t)$ and $F(t)$ represent the variation of detection efficiency and muon losses, respectively, while $B(t)$ is the background. For μ^- data the background is independent of time and at the negligible level of $\sim 2 \times 10^{-5}$, but becomes more important in the μ^+ lifetime data owing to stored proton contamination. The minimisation and measurement of these systematic effects are discussed in some detail below.

Muon losses

The muons are injected by the decay in flight of 3 GeV/c pions inflected into the ring⁴⁻⁹. They initially fill the whole available phase space; however, particle loss involves preferentially those muon orbits which approach closest to the material limits of the storage region.

To reduce these losses we manipulate the muon orbits in the first few microseconds after injection (the rotation period of the muons is 0.147 μ s) so that the muons which pass within ~ 1 cm of the walls are removed. The remaining muons are left in the central region of the aperture and have a much smaller probability of being lost. This 'scraping' is achieved by applying asymmetric voltages to the electrostatic quadrupoles¹⁰ used to focus the particles. A vertical electric field shifts the median plane downwards, and simultaneously a horizontal electric field (outwards on one side of the ring, inwards on the other) shifts the orbit sideways. The muons of large betatron amplitude then strike appropriately located aperture stops. The scraping voltages are turned off with a time constant of 10 μ s, allowing the muon population to return adiabatically to the centre of the storage region.

When a muon hits an obstacle it loses energy and has a high probability of emerging on the inside of the ring. The muon losses are therefore monitored by placing a detector against the vacuum chamber on the inside of the ring, in a position similar to that occupied by the decay electron detectors. The muon detector consists of a series of four scintillation counters each preceded by two-radiation-length lead converters. Muons are discriminated from electrons on the basis of pulse height. A 'muon' is defined as a particle which, for all four counters, gives a pulse height equivalent to that of a minimum ionising particle, the acceptance ranging from 50% below to 50% above this central value. Measurements in an electron beam show that for the relevant range of decay electron energies, this condition rejects electrons by a factor of ~ 700 . The muon detector is used to optimise the n value of the focusing field for minimum muon losses, to test the scraping system, and to measure the loss correction $F(t)$.

In the optimum running conditions very few muons were lost after 100 μ s. The above rejection ratio and the similarity in acceptance of the muon and electron detectors indicated that $\geq 80\%$ of the 'muon' counts after this time were mis-identified electrons. This was confirmed with a small five-gap optical spark chamber placed behind the muon detector. Muons,

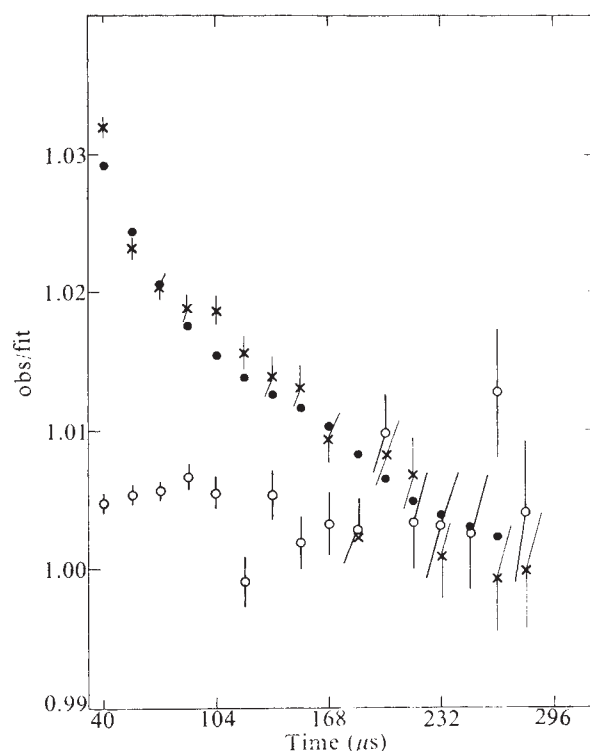


Fig. 1 Ratio of the decay electron time distribution (obs) to an exponential fit (fit) in the time range 300–650 μ s. The $g-2$ modulation is included in the fit. The excess counts at early times over the extrapolated fit function show the effect of muon losses. $\times$, obs/fit no scraping. $\bullet$, Muon loss function as calculated from the muon detector time spectrum. $\circ$, obs/fit with scraping.

identified by a straight track with four or five sparks, were found to form only 10% of the late time 'muon' counts.

The correction function $F(t)$ in equation (1) is given in terms of the lost muon time spectrum $M(t)$ (corrected for decay electron background) by the integral

$$F(t) = \frac{1}{\varepsilon \tau N_0} \int_t^{t_L} M(t) \exp(t/\tau) dt \quad (2)$$

where t_L is the end of the storage period and where the factor ε is the ratio of the detection efficiencies of the muon and electron detectors; its value and hence the absolute calibration of the muon detector is obtained as follows. In data where the losses are relatively high (those taken without scraping in operation), $F(t)$ can be deduced directly from the decay electron time distribution. Comparison with the values obtained from equation (2) provides the calibration of the muon detector and checks that it samples the lost muons in an unbiased way at different times.

This procedure and the effectiveness of the electric scraping are illustrated in Fig. 1. The points (a) represent the fractional

Table 1 Results for the muon lifetime in flight (μ s)

Run	A	B	C	D	E	F
Charge of μ	+	+	—	—	—	—
Starting time of fit	100	100	50	50	90	100
Uncorrected lifetime (statistical error)	64.406 (66)	64.295 (103)	64.444 (55)	64.336 (36)	64.366 (84)	64.278 (60)
μ -loss correction	0.008 (8)	0.008 (8)	0.013 (8)	0.012 (8)	0.009 (8)	0.008 (8)
p-background correction	0.006 (2)	0.044 (22)	—	—	—	—
Gain correction	0.022 (20)	0.016 (20)	−0.010 (20)	−0.002 (20)	0.033 (20)	0.020 (20)
Fully corrected lifetime (total error)	64.442 (69)	64.363 (108)	64.447 (59)	64.346 (42)	64.408 (87)	64.306 (64)

Table 2 Lifetime results (μs)

	μ^+	μ^-	Weighted average $\mu^+ + \mu^-$
Lifetime in flight (this experiment)	64.419 (58)	64.368 (29)	64.378 (26)
Lifetime at rest (this experiment)	2.1966 (20)	2.1948 (10)	2.1952 (9)
Lifetime at rest (previous best measurements, refs. 18, 19)	2.19711 (8)	2.198 (2)	—

excess decay electron counts at early time with respect to a fit to the unscrapped data at late time ($t > 300 \mu\text{s}$). Taking $40 \mu\text{s}$ after injection as $t = 0$, gives $F(0) = 0.03$ for these data. The points (b) are those calculated from the lost muon data, and the time dependence of these two data sets are in excellent agreement. The points (c) are the early time excess counts for the scraped data obtained in just the same way as (a). The calibrated muon detector gave a value $F(0) = 0.001$ in this case, so the loss level at $40 \mu\text{s}$ after injection is improved by a factor of ~ 30 .

For the late starting times chosen in the analysis, the muon losses contribute at most a shift of 0.02% in the lifetime, as can be seen from the entries in Table 1.

Proton background

For the μ^+ data there is another complication in that the time-varying electric field associated with scraping enables protons to be stored in the ring. (The incident π beam is unseparated.) These protons are lost with a characteristic time distribution and so give background in both the decay electron and muon detectors. By assuming that the muon losses are the same for $\mu^\pm$ with identical machine parameters, the time distribution of these lost protons can (by subtraction) be found from the counts recorded in the muon detector. The relative detection efficiency of the muon and electron detectors for these protons is established in a separate experiment where the electric field is switched off ~ 1 ms after injection. At this time ($\sim 15.5 \tau$) only protons are stored, and the ratio of counts seen in the muon and the electron detectors directly gives the efficiency ratio. It turns out that the background level in the decay electron counts due to these 'unstable' protons is low ($\sim 3 \times 10^{-3}$ at $32 \mu\text{s}$ after injection) so the resulting correction to the lifetime is small, as shown in Table 1. The contamination of the μ^- data by stored antiprotons was at a negligible level.

Gain effects

The largest systematic effect which must be considered is the change in the energy acceptance of the decay electron detection system as a function of muon storage time. This effect is due to gain changes following the injection into the ring of one or more ~ 10 ns wide bunches containing $\sim 10^7$ particles (for more details of the effect of high particle flux on the gain of photomultipliers, see ref. 14); it is minimised by blanking off the dynode chain of the photomultiplier during, and for a few microseconds after, injection.

The system gain is measured as a function of time by means of light-emitting diodes (LEDs). The injected beam and all other conditions are the same as in normal data-taking. The signals from the LEDs are distinguished from decay electron counts by timing; a series of pulses are input to LEDs on the photomultipliers of the electron detectors at a number of fixed times throughout the muon storage interval. The output signal from each electron detector is split and input to two discriminators with a threshold separation of 3 dB. The LED pulse heights are adjusted so that all pulses fire the lower threshold discriminator and $\sim 50\%$ of the pulses fire the higher one, that is, the threshold of the latter is at the maximum of the LED pulse-height spectrum where the sensitivity to gain changes is greatest. A subsidiary calibration measurement of the pulse-

height distribution for each LED-photomultiplier combination is made to establish the quantitative relation between the ratio of the counting rates output from the two discriminators and the gain change ΔG .

The gain curve is measured typically at 11 points between 7 and $580 \mu\text{s}$ for each counter, with a precision per point of $\sim 0.1\%$. From the gain curves thus obtained, a counter selection is made to remove from the analysis counters with a steep gain variation. These counters are the ones which are struck preferentially by the injected beam. The mean gain curve of the remaining counters is typically flat to within $\sim 0.1\%$ over the whole muon storage interval. The mean gain curve $G(t)$ is multiplied by the factor $S = \Delta N / (N \Delta G)$, the fractional change of counting rate of the decay electrons per unit gain change, to give the correction function in equation (1). For the electron energy threshold chosen for the lifetime analysis, $S = 1.00 \pm 0.02$.

The error in the gain correction was estimated by repeated measurement under identical running conditions. From the consistency of the slopes of mean gain curves measured at intervals of both a few days and several hours apart, this error is conservatively estimated to be $\sim 0.03\%$ in τ .

Results

Values of the muon lifetime in flight τ were found by fitting the experimental decay electron time distribution to the six-parameter function of equation (1) using the maximum likelihood method and varying the six parameters N_0 , τ , A , ω_a , ϕ , B_0 , where B_0 is the time-independent part of $B(t)$.

The results obtained for the lifetime for the two μ^+ and four μ^- runs which were separately analysed are presented in Table 1. The uncorrected lifetime, and the corrections with estimated systematic errors due to muon loss and gain effects, are shown for each run. In addition, for the μ^+ data, the proton background correction and error are shown. The starting times quoted for the fits vary from run to run as, according to the beam intensity used, the muon detector took varying times to regain full efficiency after injection. In all cases the upper limit of the time range of the fit is $650 \mu\text{s}$.

The weighted average values of the lifetimes in flight for $\mu^\pm$ together with the combined average are given in the first line of Table 2.

Fig. 2 Bunch structure in the Muon Storage Ring from 6–10 μs after injection. Each bunch corresponds to one turn in the ring. Such distributions are analysed to give the mean rotation frequency f_{rot} of the stored muons, and hence the mean γ -factor $\bar{\gamma}$.

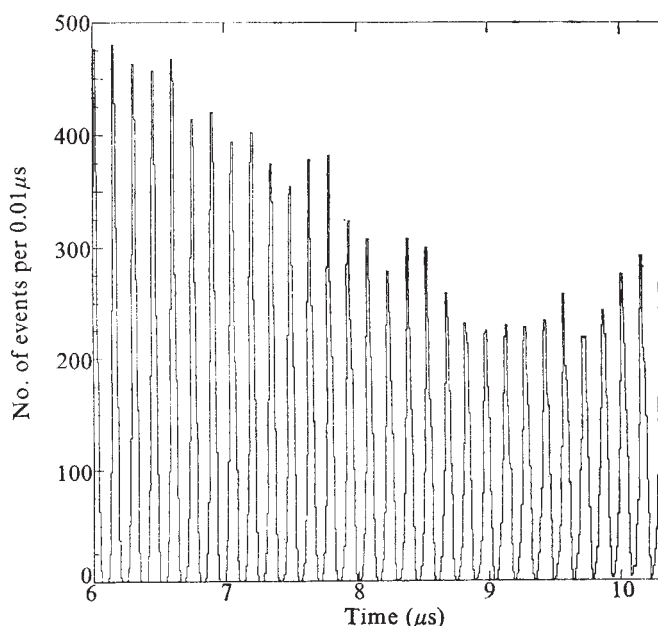


Table 3 Tests of relativistic time dilation from particle lifetime measurements. Values of $\tau_0 = \tau/\gamma$ are compared at different γ values

Particle	Refs	γ_1	γ_2	$[\tau_0(\gamma_1) - \tau_0(\gamma_2)]/\tau_0(\gamma_1)$ (%)	95% confidence limits (%)
$\pi^\pm$	19–22	1.0	2.44	-2.5 ± 0.9	–4.3 to –0.7
$K^\pm$	23, 24	1.0	3.38, 4.17	0.9 ± 0.3	0.3 to 1.5
K_s^0	25, 27	1.63	15.2	0.5 ± 0.6	–0.7 to 1.7
K_s^0	25, 26	1.63	20.2	0.2 ± 0.7	–1.2 to 1.6
μ^+	18 and this work	1.0	29.3	0.02 ± 0.09	–0.16 to 0.20

In the second line of Table 2 are given the corresponding lifetimes at rest τ_0 calculated using the Einstein² relation

$$\tau_0 = \tau/\gamma, \quad \gamma = [1 - (v/c)^2]^{-1/2}$$

The average value of γ for the circulating muons is found by analysis¹ of the bunch structure of the stored muons (see Fig. 2) over a period of $\sim 40 \mu\text{s}$ after injection. The mean rotation frequency $\bar{f}_{\text{rot}}$ is related to the average γ value $\bar{\gamma}$ by the expression

$$\bar{\gamma} = 2\lambda \bar{f}_p / g \bar{f}_{\text{rot}}$$

where $\bar{f}_p$ is the proton magnetic resonance frequency¹⁵ (corrected to vacuum¹⁶) corresponding to the mean magnetic field, g is the g -factor of the muon, and $\lambda = \mu_u/\mu_p$ is the ratio of the muon and proton magnetic moments¹⁷. The result for $\bar{\gamma}$ is found to be

$$\bar{\gamma} = 29.327 \quad (4)$$

where the quoted error corresponds to an uncertainty of $\pm 1 \text{ mm}$ in the mean radius of the distribution of circulating muons.

The last line in Table 2 gives the most accurate published values for the μ^+ (ref. 18) and μ^- (ref. 3) lifetimes measured at rest. Comparing the high precision value of the μ^+ lifetime at rest, τ_0^+ , with the value found in this experiment we obtain

$$(\tau_0^+ - \tau^+/\bar{\gamma})/\tau_0^+ = (2 \pm 9) \times 10^{-4}$$

At 95% confidence the fractional difference between τ_0^+ and $\tau^+/\bar{\gamma}$ is in the range $(-1.6-2.0) \times 10^{-3}$. To date, this is the most accurate test of relativistic time dilation using elementary particles.

This result is compared in Table 3 with other limits on the validity of special relativity calculated from lifetime measurements given in the existing literature for $\pi^\pm$ (refs 19–22), $K^\pm$ (refs 23, 24) and K_s^0 (refs 25–27).

The entries in the table are the values of, and 95% confidence limits for $[\tau_0(\gamma_1) - \tau_0(\gamma_2)]/\tau_0(\gamma_1)$, where $\tau_0(\gamma_{1,2})$ are the lifetimes at rest as calculated via the Einstein relation using measurements on particles with mean γ values $\gamma_{1,2}$. The present experiment improves on the previously existing upper limits on violations of special relativity by about an order of magnitude.

The μ^+ measurement differs in one important respect from the other results quoted in Table 3. The $\pi^\pm$, $K^\pm$ and K_s^0 measurements in flight were performed in beams for which the decaying particle, regarded as a clock, was in an inertial (unaccelerated) frame. In the muon experiment, however, the particles are subjected to a constant transverse acceleration of $10^{21} \text{ cm s}^{-2}$. The muons perform a round trip and so when compared with a muon decaying at rest in the laboratory, simulate closely the so-called twin paradox which was already discussed in Einstein's first paper². Other experiments^{28, 29} have confirmed the correctness of relativistic time dilation for clocks in circular motion to $\sim 10\%$ at low velocities (A similar conclusion follows from observations of the second-order (transverse) Doppler effect in the temperature dependence of the Mössbauer effect²³ and of the hydrogen maser frequency²⁴). The present experiment is

unique in its use of an ultra-relativistic clock ($\gamma \gg 1$) and its much greater precision $\sim 0.1\%$.

The possibility also exists that very large accelerations may modify in some way the internal constitution of particles^{32–35}. No such effects, in so far as they affect the particle lifetime, are seen in this experiment where the transverse acceleration is $\sim 10^{18} \text{ g}$.

Some authors^{36, 37} have suggested that just as special relativity breaks down at very large distances owing to gravitational effects, there may also be a breakdown below some fundamental distance α . Rédei³⁸ has calculated the effect of such a breakdown on the muon lifetime in flight, and finds a γ^2 correction to the Einstein formula

$$\tau = \gamma \tau_0 (1 + 2.5 \times 10^{24} \gamma^2 \alpha^2)$$

where α is in centimetres. From our value of τ^+ a limit can be set on α

$$\alpha \leq 9.6 \times 10^{-16} \text{ cm} \quad (95\% \text{ confidence level})$$

Assuming the correctness of special relativity, the μ^- lifetime at rest τ_0^- given in Table 2 is the most precise value so far reported. Comparing this value with the high-precision measurement of τ_0^+ it is found that

$$(\tau_0^- - \tau_0^+)/\tau_0^+ = -0.00105(46)$$

Or, at 95% confidence level, the fractional difference of τ_0^- and τ_0^+ is in the range -0.002 to -0.00013 .

The limit set on this quantity, which should vanish³⁹ by CPT invariance of the weak interaction, is comparable with that set by the best direct measurement⁸ of the ratio τ_0^-/τ_0^+ .

We thank all those who have contributed to this measurement of the muon lifetime. In particular, we thank F. Wickens for early calculations on the electric field configuration; E. M. McMillan for studies of various mechanisms of muon losses; R. W. Williams for analysis of the muon losses from the decay electron data; J. Lindsay for the preparation of a blanking scheme for photomultipliers and S. Wojcicki for his early contributions to the gain measurements.

Received 17 May; accepted 1 June 1977.

- Bailey, J. *et al.* *Nuovo Cimento* **9A**, 369 (1972).
- Einstein, A. *Ann. Phys. (Germany)* **17**, 891 (1905).
- Meyer, S. L. *et al.* *Phys. Rev.* **132**, 2693 (1963).
- Bailey, J. *et al.* *Phys. Lett.* **55B**, 420 (1975).
- Combley, F. & Picasso, E. *Phys. Rep.* **14C**, 1 (1974).
- Farley, F. J. M. *Contemp. Phys.* **16**, 413 (1975).
- Field, J. H. *Proc. EPS Internat. Conf. High-Energy Phys.* Palermo, 1975, p. 247 (Editrice Compositori, Bologna, 1976).
- Combley, F. H. *Proc. 7th Int. Symp. Lepton Photon Interactions at High Energies*, Stanford, 1975 (ed. Kirk, W. T.), p. 913 (SLAC, Stanford, 1975).
- Bailey, J. *et al.* *Phys. Lett.* **68B**, 191 (1977).
- Flegel, W. & Krienen, F. *Nuclear Instrum. Meth.* **113**, 549 (1973).
- Pizer, H. I. *Nuclear Instrum. Meth.* **123**, 461 (1975).
- Pizer, H. I., van Köningsveld, L. & Verweij, H. *CERN* 76–17 (1976).
- Field, J. H., Lange, F. & von Rüden, W. *Nuclear Instrum. Meth.* **143**, 227 (1977).
- Farley, F. J. M. & Carter, B. S. *Nuclear Instrum. Meth.* **28**, 279 (1964).
- Borer, K. *Nuclear Instrum. Meth.* **143**, 203 (1977).
- Borer, K. & Lange, F. *Nuclear Instrum. Meth.* **143**, 219 (1977).
- Crowe, K. M. *Phys. Rev. D* **5**, 2145 (1972).
- Balandin, M. P., Crebenyuk, V. M., Zinov, V. G., Konin, A. D. & Ponomarev, A. N. *Soviet Phys. JETP* **40**, 811 (1974).
- Nordberg, M. E., Lobkowicz, F. & Burman, R. L. *Phys. Lett.* **24B**, 594 (1967).
- Ayres, D. S. *et al.* *Phys. Rev. D* **3**, 105 (1971).
- Dunaitsev, A. F., Prokoshkin, Y. D., Razuvaev, E. A., Sergeev, V. A. & Simonov, Y. N. *Soviet J. Nuclear Phys.* **16**, 292 (1973).

- ²² Ayres, D. S. *et al.* *Phys. Rev.* **157**, 1288 (1967).
²³ Ott, R. J. & Pritchard, T. W. *Phys. Rev. D* **3**, 52 (1971).
²⁴ Lobkowicz, F., Melissinos, A. C., Nagashima, Y. & Tewksbury, S. *Phys. Rev.* **185**, 1676 (1969).
²⁵ Skjeggstad, O. *et al.* *Nuclear Phys.* **48B**, 343 (1972).
²⁶ Geweniger, C. *et al.* *Phys. Lett.* **48B**, 487 (1974).
²⁷ Carithers, W. C. *et al.* *Phys. Rev. Lett.* **34**, 1244 (1975).
²⁸ Hay, H. J., Schiffer, J. P., Cranshaw, T. E. & Egelstaff, P. A. *Phys. Rev. Lett.* **4**, 165 (1960).
²⁹ Hafele, J. C. & Keating, R. E. *Science* **177**, 166 (1972).
³⁰ Pound, R. V. & Rebka, G. A., Jr *Phys. Rev. Lett.* **4**, 274 (1960).
³¹ Vessot, R. F. C. & Levine, M. W. *Metrologia* **6**, 116 (1970).
³² Sherwin, C. W. *Phys. Rev.* **120**, 17 (1960).
³³ Romain, J. E. *Rev. mod. Phys.* **35**, 376 (1963).
³⁴ Bailey, J. & Picasso, E. *Prog. Nuclear Phys.* **12**, 43 (1970).
³⁵ Ageno, M. & Amaldi, E. *Accademie Nazionali dei Lincei, Serie VIII, VIII, Fascicolo I* (1966).
³⁶ Blokhintsev, D. I. *Phys. Lett.* **12**, 272 (1964).
³⁷ Rédei, L. B. *Phys. Rev.* **145**, 999 (1967).
³⁸ Rédei, L. B. *Phys. Rev.* **162**, 1299 (1967).
³⁹ Lüders, G. & Zumino, B. *Phys. Rev.* **106**, 345 (1957).

Rifting and subsidence on passive continental margins in the North East Atlantic

L. Montadert

Division Geologie, Institut Français du Pétrole, 1.4 Av. de Bois-Preau, 92502 Rueil Malmaison, France

D. G. Roberts

Institute of Oceanographic Sciences, Wormley, Godalming, Surrey, UK

G. A. Auffret

Centre Oceanologique de Bretagne, CNEXO, 29.273 Brest, France

W. Bock

Rosentiel School of Marine & Atmos. Science, 4600 Rickenbacker Causeway, Miami, Florida 33149

P. A. DuPeuble

Université de Rouen, Laboratoire de Géologie, Faculté des Sciences, 76130 Mont Saint-Aignan, France

E. A. Hailwood

Department of Oceanography, University of Southampton, Southampton, UK

W. Harrison

Oklahoma Geological Survey, University of Oklahoma, 830 van Fleet Oval, Norman, Oklahoma 73069

H. Kagami

Ocean Research Institute, University of Tokyo, Nakano, Tokyo 164, Japan

D. N. Lumsden

Department of Geology, Memphis State University, Memphis, Tennessee 38152

C. Muller

Geologisch-Paläontologisches Institut, Johann-Wolfgang-Goethe-Universität, Frankfurt, Germany

D. Schmitker

Department of Oceanography, University of Maine, Walpole, Maine 04573

R. W. Thompson

Humboldt State University, School of Natural Resources—Oceanography, Arcata, California 95521

T. L. Thompson

University of Oklahoma, Department of Geology & Geophysics, Norman, Oklahoma 73069

P. P. Timofeev

Geological Institute, USSR Academy of Sciences, Pyzhevsky per 7, Moscow ZH 17, USSR

Deep-sea drilling in the Bay of Biscay and the Rockall Plateau show that passive continental margins are characterised by the absence of seismicity and include continental shelf, continental slope and continental rise physiographic provinces, exemplified by the borders of the Atlantic Ocean.

HYPOTHESES postulated for the structural and stratigraphical evolution of passive margins are based on the available geological and geophysical data, and analogies drawn with present day margins thought to represent stages in margin evolution¹⁻⁵. In the initial stages, rifting of the continent perhaps contemporaneous with regional uplift takes place⁶. When the continental crust is completely rifted apart, ocean crust begins to accrete at the axis of the diverging plates. Heat dissipation⁷ through the cooling continental and oceanic lithosphere is thought to result in subsidence, permitting transgression. The subsequent development of the margin depends on the variable roles in both time and space of subsidence, sediment loading, climate and ocean circulation. For example, the lithology and volume of sediments may be influenced by global transgressions and regressions^{8,9}, although their effects, in turn, depend on the altitudes of the continents and their rate of subsidence.

The relationship between the subsidence history, the rifting environment and the unconformities observed beneath many passive margins is, in particular, poorly understood because of a paucity of relevant deep sea drilling data. We report here some preliminary relevant results from the holes

drilled on the passive margins of the Bay of Biscay and the Rockall Plateau during Leg 48 of the International Phase of Ocean Drilling (IPOD) of the Deep Sea Drilling Project (Figs 1, 2, 3).

The Bay of Biscay and Rockall Plateau

The northern margin of the Bay of Biscay¹⁰⁻¹⁴ is comprised of the wide Celtic shelf and a broad continental slope that is dissected by many canyons and broken by the Meriadzek Terrace. Detailed multi-channel seismic surveys made by the Institut Français du Pétrole, the Institute of Oceanographic Sciences and the Centre National d'Exploitation des Océans, show thin Tertiary and Cretaceous sediments overlying tilted blocks and half grabens that trend sub-parallel to the margin and control the relief of the Meriadzek Terrace (Fig. 2). Previous geological and geophysical studies have suggested that the Bay of Biscay was formed by rifting in Late Jurassic–Early Cretaceous time and by spreading during Early to Upper Cretaceous time¹⁰⁻¹⁸. However, the relation between the spreading and the subsidence history of the margin is not known. The three sites drilled in Biscay (Fig. 2) form a transect from the upper slope (site 402A) to the lower slope (site 400A) within 40 km of the continent–ocean boundary at the Trevelyan escarpment¹⁸.

In contrast to the Bay of Biscay, three phases of rifting and spreading isolated the Rockall Plateau microcontinent and formed its margins¹⁹⁻²³. The first opened the Rockall Trough in Early–Late Cretaceous time, and the second, in Late Cretaceous time (76 Myr), opened the Labrador Sea,

thus spreading the Greenland–Rockall plate away from North America. In the final phase, rifting and volcanism at about 60 Myr were followed by spreading beginning at 52–53 Myr between Greenland and Rockall. Sites 403 and 404 were drilled in a broad fault-bounded basin some 30 km east of the oldest magnetic anomaly²⁴ (52–53 Myr), recorded in the adjacent ocean crust. Within the basin, a thick, faulted sequence of deltaic aspect is unconformably overlain by a thin unfaulted sequence (Fig. 2). Sites 405 and 406 were drilled on ocean crust south of the east–west trending transform fault controlling the south-west margin

suggests a depth of 1,000 to 1,500 m. The Pliocene and Pleistocene foram nanno oozes were apparently deposited at the present depth.

At site 401 (Fig. 1, Fig. 3), located on the edge of the Meriadzek Terrace in 2,555 m depth, the oldest sediments encountered were Jurassic (Kimmeridgian–Portlandian) limestones containing echinoderm and pelecypod debris, algal and coral fragments, indicative of a shallow-water reefal environment. Tithonian calpionellid limestones have been dredged nearby²⁴. An overlying limestone of Lower Cretaceous age (Fig. 3) contained algae, bryozoa, corals and echinoderms. Nearby dredge hauls have yielded reefal limestones of Berriasian, Neocomian and Aptian age²⁴. The overlying Campanian chalk contains abundant *Stilostomella*, *Gyroidina*, *Cibicidoides*, etc., indicating a depth of deposition of at least 1,500 m, while a lower bathyl depth is indicated for the Lower and Upper Palaeocene marly nanno chalks. The Mid-Eocene assemblages suggest site 401 was then at, or close to, its present depth²⁵.

At site 400A (Fig. 1, Fig. 3), the Aptian planktonic faunas found in the carbonaceous marly chalks are oceanic

Fig. 2 a, Location of IPOD–DSDP Leg 48 holes on the south-west Rockall Plateau. b, Tracing of multichannel seismic section through sites 403 and 404.

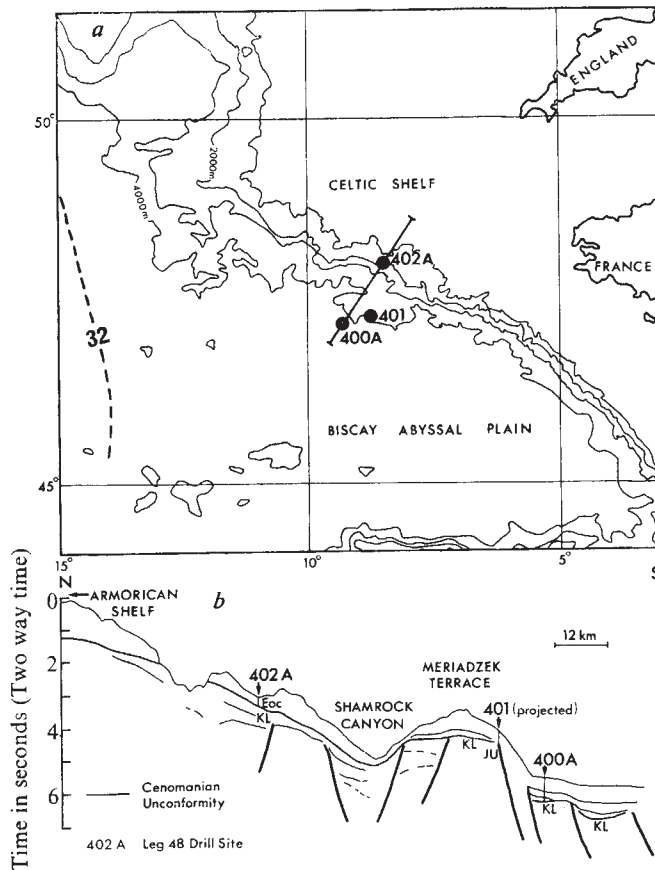


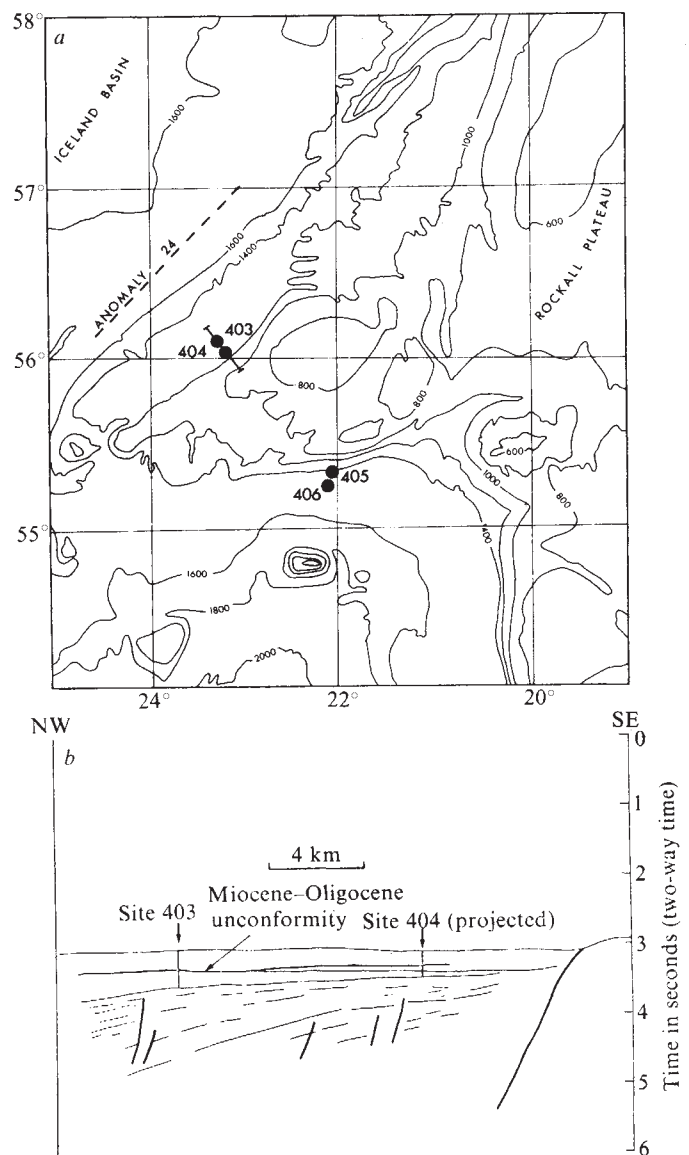
Fig. 1 a, Location of IPOD–DSDP Leg 48 holes in the Bay of Biscay. 32–location of magnetic anomaly-32(76 Myr). b, Schematic cross section of the margin. Drillsite locations shown as 402A.

of the Rockall Plateau, and will not be discussed here. The generalised lithology and stratigraphy of the holes drilled during Leg 48 are shown in Fig. 3.

Palaeobathymetry

The palaeobathymetric history of the sites has been inferred principally from the macro- and microfossil assemblages, the associated lithologies, and seismic profiles which show the absence of post-rift faulting.

At site 402A (Fig. 1, Fig. 3), now situated on the upper slope of Biscay in 2,317 m depth, the Aptian carbonaceous limestones contain rare radiolaria and benthic foraminifera such as *Nodosaria* sp., *Lenticulina* sp. and *Epistomina spinulifera* indicative of a shallow shelf sea. The microfacies of the Albian limestones, as observed in thin section, are closely similar to the epicontinental Albian of the Aquitaine Basin (P. A. Du Peuble, unpublished data). In contrast, the Middle and Late Eocene nannofossil chalks contain deep-water species such as *Stilostomella* sp. However, shallow-water species, that may be displaced, are common and include *Bolivina* and *Rosalina*. The deep-water fauna



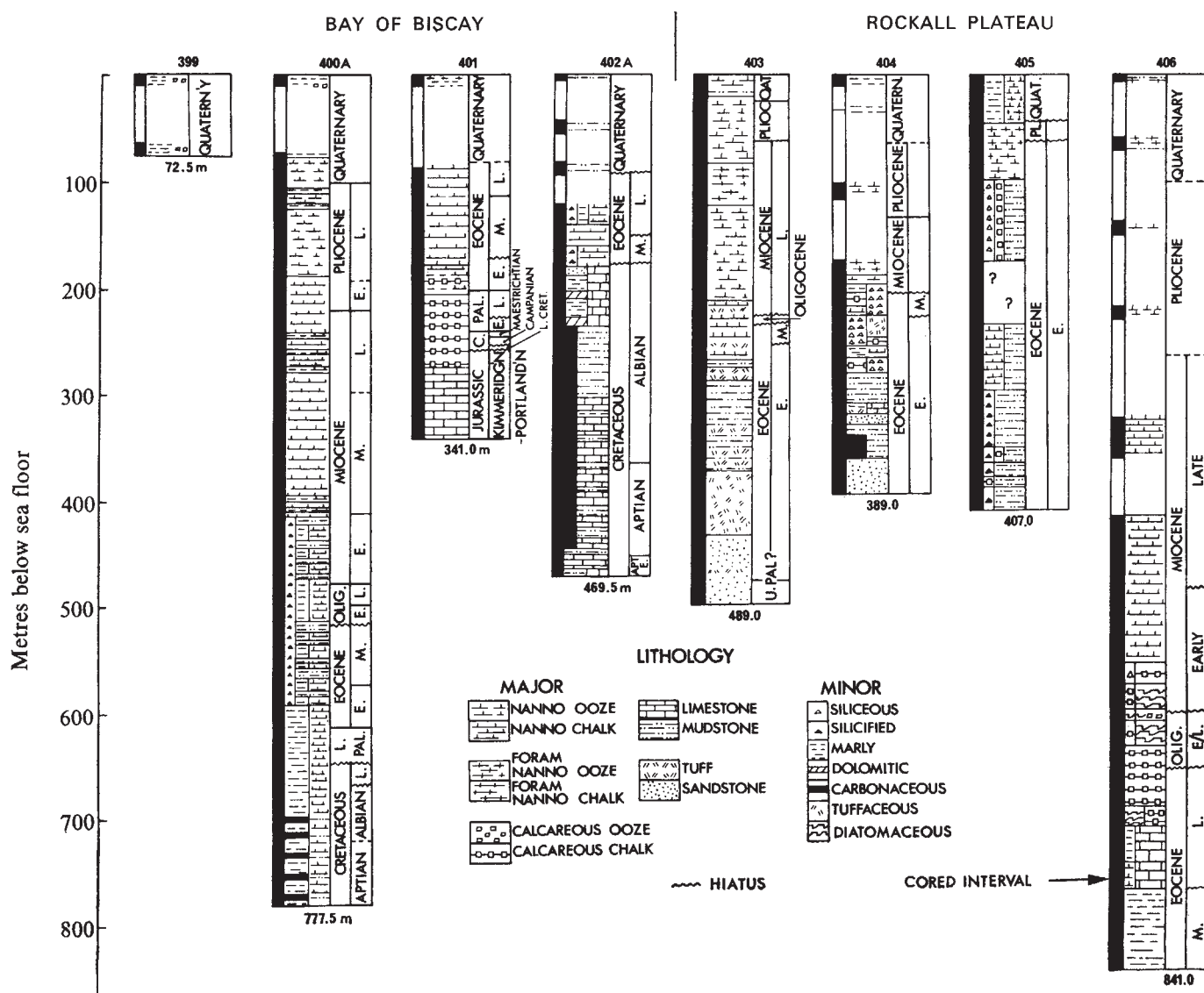
and show evidence of dissolution. The Aptian benthic foraminifera include deep-water species such as *Pleurostomella subnodosa*, *Gyrodinioides primitiva*, *Glomospira* sp. and others. The Late Cretaceous fauna has been severely affected by dissolution, and benthic foraminifera are rare though one species, *Aragonia trinitatis* is a known deep-water form²⁵. An abyssal realm of deposition for the Palaeocene claystones and marly chalks is indicated by the benthic foraminifera *Anomalina* sp., *Gavelinella* sp. and *Glomospira gordialis*. The Eocene and Oligocene foraminiferal assemblage is nearly identical to that described from the Oceanic Formation of Barbados and considered to have been deposited in 4–5,000 m depth²⁶. Although these data show a progressive increase in depth since Aptian time, quantitative depth inferences are not possible due to poor preservation and the difficulty of assigning precise depth ranges to abyssal benthic faunas. Approximate palaeodepths for the site can be derived, however, from the seismic profiles in the vicinity of sites 400A and 401. These profiles show insignificant post-Aptian faulting, thus indicating that the present 2,000 m of relief between sites 400A and 401 had been created by Aptian time. The relative elevation of the Albian beds at these sites, less a small correction for the Albian palaeodepth at site 401, gives an approximate

palaeodepth of site 400A in Albian time of 2,200 m. Using the same method, the Upper Cretaceous and Tertiary palaeodepths of site 401 have been transferred to site 400A and give palaeodepths consistent with the faunal evidence of contemporaneous abyssal depths at site 400A.

The 'Cenomanian' hiatus found at site 400A does not indicate subaerial erosion and uplift, for deep-water Campanian chalks rest directly on deep-water carbonaceous limestones of Albian age without evidence of intra Albian-Campanian faulting (Fig. 1, Fig. 3). At sites 401 and 402A, where the same hiatus is present, subaerial erosion and uplift are again precluded by the absence of post-Albian faulting. The missing Eocene-Lower Cretaceous and Campanian-Lower Cretaceous sections at these sites were either not deposited or were removed by erosion, perhaps by more vigorous bottom currents associated with the 'Cenomanian' transgression.

At Sites 403 and 404 (Fig. 2), on the Rockall Plateau the sequence becomes increasingly clastic downsection. Site 404 bottomed in a conglomerate containing oyster shells that suggests a littoral environment (Fig. 3). The overlying Lower Eocene contains a scarce fauna similar to the Lower Eocene 'London Clay' formation deposited in shallow water. Above 352 m deep-water species were not found, but

Fig. 3 Generalised lithology and stratigraphy of sites drilled during DSDP Leg 48.



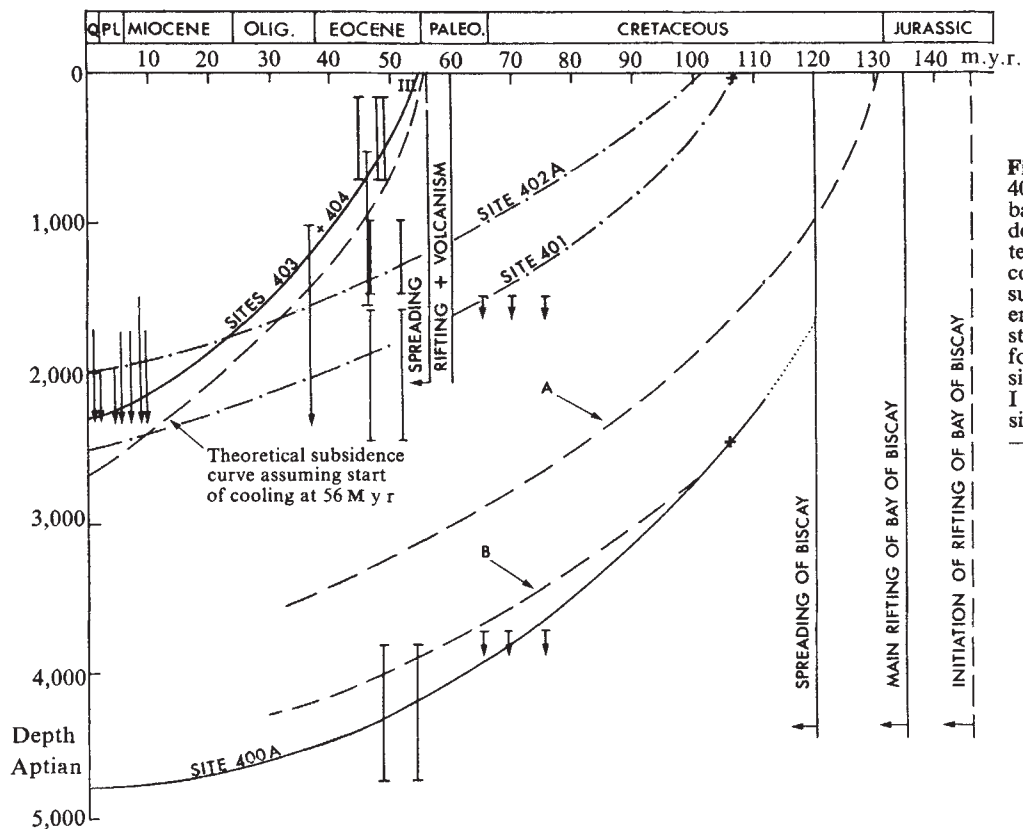


Fig. 4 Subsidence curves for sites 400A, 401, 402/402A, 403 and 404. Vertical bars indicate palaeobathymetric data derived from micropalaeontological criteria. *a*, Subsidence curve for start of cooling at 130 Myr. *b*, Fit of theoretical subsidence curve to observed subsidence curve of site 400A assuming start of cooling at 120 Myr. The curve for site 400A is based on data from sites 401 and 402A in addition. ↓ I Palaeodepth range. —, Subsidence curve sites 401 and 402A. —, Subsidence curve sites 403 and 404.

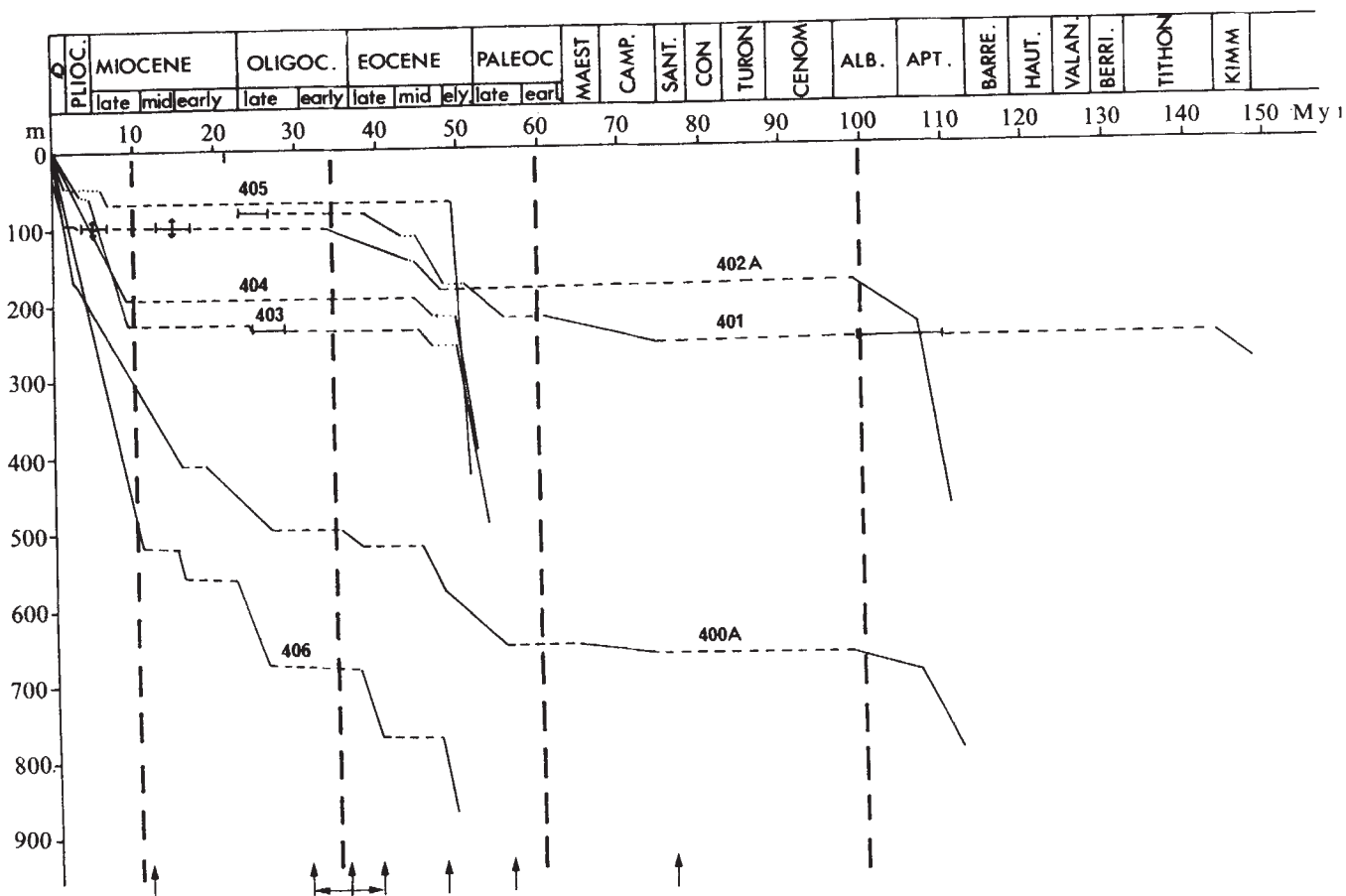


Fig. 5 Sedimentation rate curves for sites drilled in Leg 48. Changes in spreading rate and/or direction after refs 32, 33 and 34. (--- indicates unconformities recorded on the adjacent shelf of NW Europe). ↑, Change in spreading and/or direction.

abundant plano-convex *Cibicides* indicative of shallow water are present. An upper bathyal depth during the Middle Eocene is suggested by an assemblage of bathyal and upper slope species. During the Late Miocene to the present, lower bathyal depths are indicated by the benthic foraminifera.

Rifting, spreading and subsidence

The subsidence curves given in Fig. 5 describe the depth of the sea floor at the drill sites as a function of age and are compared with theoretical age against depth curves for the Pacific Ocean crust. The curve for site 400A, however, describes the depth of the top of the Aptian as a function of age. The margin subsidence curves have not been isostatically corrected for sediment loading in view of the small sediment thicknesses at each site. In the case of site 403, the isostatically corrected depth is 2,407 m and in the case of site 400A, 4,626 m. These minor corrections further reduce the already small difference in depth between the two sets of curves.

The close similarity of the two sets of curves suggests that these passive margins have thermally subsided with a time constant that may be similar to the 50 Myr exponential time constant deduced for the ocean crust^{7,27}. Although the law of subsidence thus seems to be similar, the age-versus-depth relationship for the ocean crust cannot, however, be validly extrapolated to a subsiding passive margin. This is because the absolute depth of a point on a subsiding margin depends on its initial altitude relative to sea level and not on its age, as is the case for the ocean crust. Clearly, points initially above sea level will seem to have subsided less than those below.

In understanding the application of these subsidence curves to passive margins, it is important to distinguish clearly between the thermal régimes of rifting and spreading. During rifting, the continental crust is extended but remains joined so that the heat source remains fixed beneath the rift axis. In contrast, during spreading, the heat source migrates away from the passive margins as new crust accretes at the ridge axis. Cooling of the margin can only begin at the end of rifting and the onset of spreading.

If the preceding discussion is correct, the curves can be used to infer both the onset of spreading and the palaeo-depth of the margin at that time. In the case of the Rockall Plateau, extrapolation of the observed curve backward in time and comparison with the theoretical cooling curve suggests initiation of cooling at about 55 Myr. This date closely agrees with the onset of spreading at 52–53 Myr given by the age of the oldest magnetic anomaly²² recorded in the adjacent ocean crust²⁸. The curve also shows that the site was in inner shelf depths at the onset of spreading and cooling.

In the case of the Bay of Biscay, theoretical curves that assume initiation of cooling at 130 Myr for a site at sea level, lie deeper than the curves for sites 401 and 402A and shallower than that for site 400A. The closest correspondence between observed and theoretical curves for site 400A suggests initiation of cooling at about 120 Myr with the site already in 2,000 m depths. Subject to improved palaeobathymetric data, these curves suggest that spreading was initiated in 2,000 m water depths at about 120 Myr in the Bay of Biscay. This conclusion is independently supported by seismic reflection profiles which show that the Albian–Aptian carbonaceous limestones rest on the oceanic crust of the Bay of Biscay.

Although the relationship between subsidence and spreading seems the same for Biscay and Rockall, there are important and unexpected differences in the rifting and post-rifting environments. On the Rockall Plateau, and in Greenland, rifting was associated with volcanism and probably with a substantial subaerial relief, for the first post-rift sediments were deposited in inner shelf conditions

at the foot of a 1,000-m scarp close to the continent–ocean boundary (Fig. 2). In Biscay, the Late Jurassic–Early Cretaceous rifting was apparently associated with minor volcanism^{12,29} and produced some 2,000 m of submarine relief by the time the first post-rift sediments were laid down. We tentatively suggest that these differences may reflect rifting of an emerged Pre-Cambrian craton in the case of Rockall Plateau and, in the case of Biscay, rifting of an epicontinental Jurassic basin underlain by crust perhaps weakened during earlier Triassic rifting. The results suggest that the subsidence law for passive margins is independent of the structure and initial altitude of the continent and that passive margins begin to subside at the onset of spreading. The subsidence of both margins was accompanied by a gentle oceanward tilt of the margin rather than renewed faulting (Fig. 1, 2). However, the subsidence may have been discontinuous since hiatuses were found on the margins of Biscay and Rockall (Fig. 6). The origin and nature of such hiatuses in both the deep-sea geological record and the adjacent margins is not well understood. The reasonable correlation (Fig. 6) between the hiatuses and/or sedimentation rate changes observed on Biscay and Rockall surely implies the importance of large scale correlative events. In the deep-sea geological record, such events have usually been related to changes in ocean circulation arising from plate reorganisation and/or changes in the sill depths of the ocean basins³⁰. In both Biscay and Rockall, increased sedimentation seems to follow spreading rate changes at 55 Myr and 10 Myr, though the correlation is less clear for the Oligocene–Eocene change in spreading. A more surprising correlation seems to exist between these events and unconformities recorded on the adjacent shelf and land (Fig. 6) and associated with uplift^{24,31}. If this correlation is real, and validated by further deep-sea drilling, it is important and puzzling for it implies that the continental part of a plate responds vertically in coincidence with changes in spreading rate.

Received 18 February; accepted 25 May 1977.

- 1 Heezen, B. C. *Scient. Am.* 203, 98–110 (1960).
- 2 Roberts, D. G. & Caston, V. N. D. in *Proc. 9th World Petrol. Congress* 2, 281–298 (Applied Science Publishers, London, 1975).
- 3 Falvey, D. A. *Austral. Petrol. Expl. Ass. J.* 12, 86–88 (1972).
- 4 Lowell, J. D. & Genik, G. J. *Am. Ass. petrol. Geol. Bull.* 56, 247–259 (1972).
- 5 Schneider, E. D. in *Studies in Earth and Space Sciences*, (eds R. Shagam et al. (Geol. Soc. Am. Mem. Harry H. Hess Volume 132, 109–118, 1973).
- 6 Saggerson, E. P. & Baker, B. H. *Geol. Soc. Lond. Quart. J.* 121, 51–72 (1965).
- 7 Sleep, N. *Geophys. J. R. Astron. Soc.* 24, 325–350 (1971).
- 8 Hays, J. D. & Pitman, W. C. *Nature* 246, 18–22 (1973).
- 9 Flemming, N. C. & Roberts, D. G. *Nature* 243, 19–22 (1973).
- 10 Montadert, L., Damotte, B., Debysier, J., Fail, J. P., Delteil, J. R. & Valery, P. *Geology of the East Atlantic Margin* 43–74 (1971).
- 11 Montadert, L., Damotte, B., Delteil, J. R., Valery, P. & Winnock, E. *Paper III 2, in Histoire Structurale du Golfe de Gascogne* 1 (Editions Technip, Paris, 1971).
- 12 Montadert, L. & Winnock, E. *Paper VI 16 in Histoire Structurale du Golfe de Gascogne* 2 (Editions Technip, Paris, 1971).
- 13 Montadert, L., Winnock, E., Delteil, J. R. & Grau, G. in the *Geology of Continental Margins* 323–342 (Springer-Verlag, New York, 1974).
- 14 Laughton, A. S., Roberts, D. G. & Graves, R. *Deep-sea Res.* 22, 791–810 (1975).
- 15 Le Pichon, X., Bonnin, J., Francheteau, J. & Sibuet, J.-C. *Paper VI 11 in Histoire Structurale du Golfe de Gascogne* 2 (Editions Technip, Paris, 1971).
- 16 Williams, C. A. *Nature* 244, 86–88 (1973).
- 17 Jones, M. T. & Roberts, D. G. *Abstr. Mtg of European Geol. Soc. Reading* (Geol. Soc., London, 1975).
- 18 Bacon, M. & Cray, F. *Nature* 229, 331–332.
- 19 Laughton, A. S. *Nature* 232, 612–616.
- 20 Le Pichon, X., Hyndman, R. & Pantot, G. *J. geophys. Res.* 76, 4724–4743.
- 21 Vogt, P. R. & Avery, O. E. *J. geophys. Res.* 79, 363–389 (1974).
- 22 Roberts, D. G. in *The Geology of Continental Margins* 343–360 (Springer-Verlag, New York, 1974).
- 23 Roberts, D. G. *Phil Trans. R. Soc.* 278, 447–509 (1975).
- 24 Pastouret, L. & Auffret, G. *Revue de l'Institut Français du Pétrole* 31, 401–425 (1976).
- 25 Douglas, R. G. *Initial Reports of the Deep Sea Drilling Project Leg XVII* 673–694 (1973).
- 26 Beckmann, J. P. *Eclogae, Geol. Helvet* 46, 301–412 (1953).
- 27 Slater, J. G., Anderson, R. N. & Bell, M. L. *J. geophys. Res.* 76, 7888–7915 (1971).
- 28 Soper, N. J., Downie, C., Higgins, A. C. & Costa, L. I. *Earth planet. Sci. Lett.* 32, 149–157 (1976).
- 29 Cowperthwaite, I. A., Fitch, F. J., Miller, J. A., Mitchell, J. G. & Robertson, R. H. S. *Clay. Mineral* 9, 309–327 (1972).
- 30 Davies, T. A., Weser, O. E., Luyendyk, B. P. & Kidd, R. B. *Nature* 253, 15–19 (1975).
- 31 Curry, D., Hamilton, D. & Smith, A. J. in *The Geology of the East Atlantic Continental Margin* 2, 129–142 (Inst. Geol. Sci. Rept., 70114, 1971).
- 32 Williams, C. A. & Mackenzie, D. P. *Nature* 232, 168–173 (1971).
- 33 Vogt, P. R., Johnson, G. L., Holcombe, T. L., Gilg, J. G. & Avery, O. E. *Tecto-no physics* 12, 211–234 (1971).
- 34 Vogt, P. R. & Avery, O. E. *J. geophys. Res.* 79, 363–389 (1974).

Coupling of hormone receptors to adenylate cyclase of different cells by cell fusion

M. Schramm, J. Orly, S. Eimerl & M. Korner

Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel

A new hormone responsiveness can be conferred on an adenylate cyclase system by coupling it to a foreign hormone receptor. Receptor and enzyme from cells of different species can be compatible with each other.

In recent years, the fusion of different cells has been intensively studied¹⁻⁴. By this process the components of the cell membrane of two different cells could be intermixed^{1,5,6}. We have decided to use this phenomenon for the analysis of complex biochemical systems residing in the membrane. Thus, one could test whether a hormone receptor, or enzyme or carrier function is a dissociable unit which can couple to the homologous machinery of another type of cell. Employing the fusion process, we have recently shown that the catecholamine receptor of turkey erythrocytes is able to activate the adenylate cyclase of mouse erythroleukaemia cells (F cells)⁷.

The turkey erythrocytes were first treated with *N*-ethylmaleimide (NEM) or heat to inactivate their adenylate cyclase but not their catecholamine receptor. The F cells possessed an adenylate cyclase but no catecholamine receptor. Fusion of the two cell types with each other by Sendai virus resulted in coupling of the hormone receptor from one cell with the enzyme from the other cell. Inhibitors of protein synthesis had no effect on the process and it was, therefore, concluded that the coupling occurred between pre-existing components.

The experiment established that the catecholamine receptor is a component which is independent of the catalytic adenylate cyclase unit and that the receptor is compatible with the enzyme of another cell. However, the possibility could not be excluded that the catecholamine receptor of the erythrocyte was compatible with the enzyme of the F cell only, because both cells are of erythropoietic origin. There was also no information whether any other hormone receptor could be transferred to a heterologous system.

Present studies show that the β -adrenergic receptor can be donated and coupled to heterologous adenylate cyclase systems among a number of cell types of diverse origin and cellular properties. The prostaglandin E1 receptor can also be transferred to adenylate cyclase systems of other cells. Conclusions, with regard to the nature of hormone receptors which couple to adenylate cyclase, are discussed.

Transfer of the β -adrenergic receptor

The NEM-treated erythrocytes (E_{NEM}) bearing the catecholamine receptor, readily and massively fused with mouse adrenal tumour cells (Y-1) (ref. 8). Cell ghosts prepared immediately after fusion, showed a fivefold activation of adenylate cyclase by isoproterenol (Fig. 1). A control fusion of the Y-1 cells among themselves did not produce a catecholamine-responsive system. In addition, binding¹⁸ studies showed that the Y-1 cells contained no measurable amounts of catecholamine receptor. It was concluded that activation by the catecholamine was dependent on the transfer of the receptor from the turkey erythrocytes to

the Y-1 cells. In this study, basal activity of adenylate cyclase and activity in presence of fluoride were affected to some extent by the fusion process. However, this did not interfere with interpretation of the experiments since the specific hormonal response is measured as the increase over basal activity. Additional experiments showed that

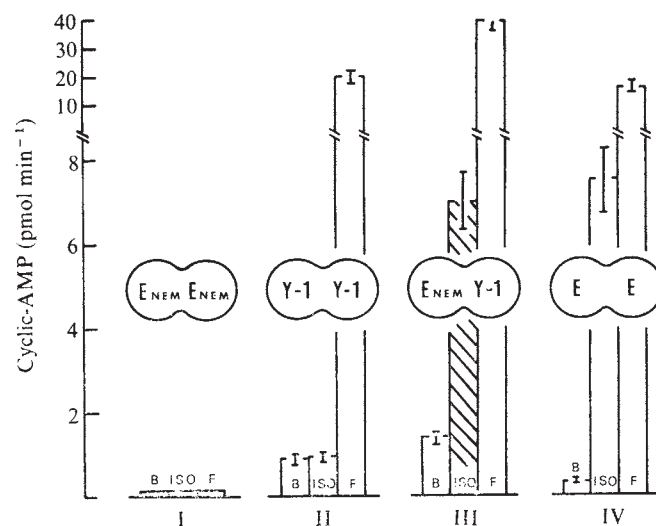


Fig. 1 Coupling of the β -adrenergic receptor of turkey erythrocytes to adenylate cyclase of rat adrenal Y-1 tumor cells by cell fusion. Adenylate cyclase activities are shown for cell ghosts prepared after fusion⁷. Systems were: (i) NEM-treated erythrocytes (E_{NEM}), 3.2×10^8 cells fused with each other; (ii) Y-1 cells, 3.0×10^8 fused with each other; (iii) heterologous fusion of E_{NEM} cells, 3.2×10^8 with Y-1 cells, 3.0×10^8 ; (iv) untreated erythrocytes (E), 3.2×10^8 fused with each other. The cell types which participate in fusion are symbolised in the figure by fused circles in each set of columns. At the base of each column the activating agent is specified: B, basal activity in the presence of $20 \mu M$ propranolol; ISO, $50 \mu M$ isoproterenol; F, $10 mM$ fluoride. Bars at the top of the column show the range of activities of duplicate fusion systems. The response of the hormone receptor transferred by fusion to a heterologous system is emphasised by a hatched column. Adenylate cyclase activity⁹ shown was measured in duplicate on cell ghosts equivalent to 1/7 of the cells in the fusion system. Y-1 cells (ATCC) were grown in Roux bottles and were subcultured as described⁸. Cells from confluent cultures (5–10 d) were suspended as follows; the monolayers of cells were washed with phosphate-buffered saline (PBS) pH 7.5 (140 mM NaCl, 20 mM KCl 10 mM KH_2PO_4). Two ml of PBS containing trypsin, 0.01% (w/v) (Worthington $2 \times$ crystallised and lyophilised) chymotrypsin, 0.01% (w/v) (Sigma $3 \times$ crystallised) and EDTA, 0.7 mM were placed on the cultures. After incubation for 3–5 min at $37^\circ C$, trypsinisation was terminated by addition of 10 ml growth medium containing Soybean trypsin inhibitor (STI), 1 mg ml⁻¹. The cells were washed twice in Na^+ salt medium⁷ and resuspended for fusion in Na^+ salt medium containing STI (0.5 mg ml⁻¹). Turkey erythrocytes were prepared for fusion as previously described⁷. The procedure for fusion by Sendai virus was adapted⁷ from that of Loyer *et al.*⁴. Sendai virus was produced¹⁰ by Dr Loyer of this department. The amount of virus was 3,000 HAU in systems i, iii, iv and 2,000 HAU in system ii. Progress of fusion was followed by phase contrast microscopy. After 45 min at $37^\circ C$ when most of the Y-1 cells were fused with erythrocytes, the cells were transferred to ice and cell ghosts were prepared by hypotonic lysis⁷. Experiments were repeated at least three times.

C6 (BU1) rat glioma cells^{11,12} treated with NEM readily transferred their catecholamine receptor to F cells (Fig. 2). These findings showed that neither the erythrocytes nor the F cells used in our previous work are unique partners for the coupling experiment. A successful matching of the β -adrenergic receptor of C6 cells with the adenylate cyclase of Y-1 cells was therefore predicted and demonstrated (Fig. 3). The presence of protein synthesis inhibitors before and during Y-1 self fusion had no effect on transfer of the β -adrenergic receptor. It is, therefore, obvious that the coupling occurred between pre-existing components.

The question whether the affinity of the β -adrenergic receptor for agonists is affected by the milieu in which it operates is of considerable interest^{13,14}. The ability to couple the receptor to an adenylate cyclase of another cell presented a unique opportunity to submit the problem to actual testing. It is shown in Table 1 that the dissociation constants for adrenaline, noradrenaline and propranolol do not change significantly when the β -adrenergic receptor of the erythrocytes becomes coupled to the adenylate cyclase of F cells or Y-1 cells. It is particularly noteworthy that the ratio of dissociation constants for noradrenaline-adrenaline, which serves to distinguish various types of β -adrenergic receptors¹⁵, remains essentially unchanged.

In a further experiment rat hepatoma cells (HTC) which have no adenylate cyclase activity¹⁶ produced a response to isoproterenol when fused with F cells. Stimulation of adenylate cyclase over basal activity was only 1.8-fold but was consistently obtained in three independent experiments (data not shown). Surprisingly, this indicates that a receptor without apparent function had been preserved throughout many months of growth of the HTC cells in suspension culture. A selected variant of HTC, grown in stationary culture, has recently been shown to bind a β -adrenergic antagonist¹⁸.

Transfer of the prostaglandin E1 receptor

The experiments on the β -adrenergic receptor suggested that perhaps other hormone receptors could also be trans-

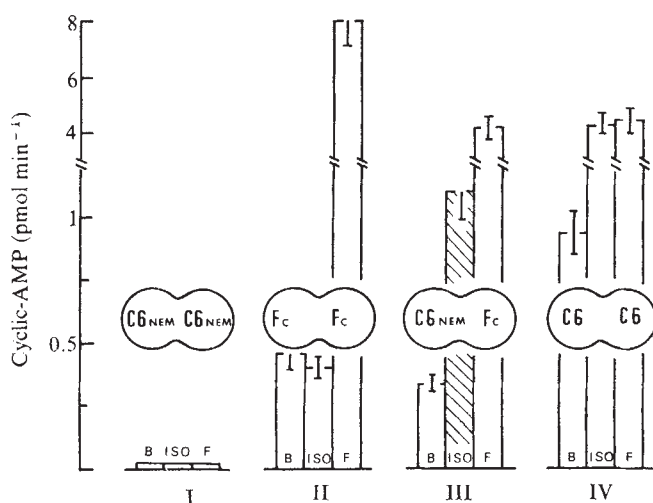


Fig. 2 Coupling of the β -adrenergic receptor of rat glioma cells (C6) with adenylate cyclase of Friend erythroleukaemia (Fc) cells. Systems were: (i) NEM-treated C6 cells ($C6_{NEM}$), 1.5×10^6 , fused with each other; (ii) F cells, 1.2×10^6 fused with each other; (iii) heterologous fusion between $C6_{NEM}$ cells, 1.5×10^6 and F cells, 1.2×10^6 ; (iv) untreated C6 cells, 1.5×10^6 , fused with each other. Other details are as in Fig. 1. F cells were grown and collected for fusion as previously described⁷. C6 cells were grown and collected for fusion as described for Y-1 cells in Fig. 1. (The culture was given by Dr U. Bachrach of this university.) The amount of virus was 400 HAU in systems i, iii and 200 HAU in ii and iv. After 22 min at 37 °C the systems were transferred to the cold and cell ghosts were prepared by hypotonic lysis.

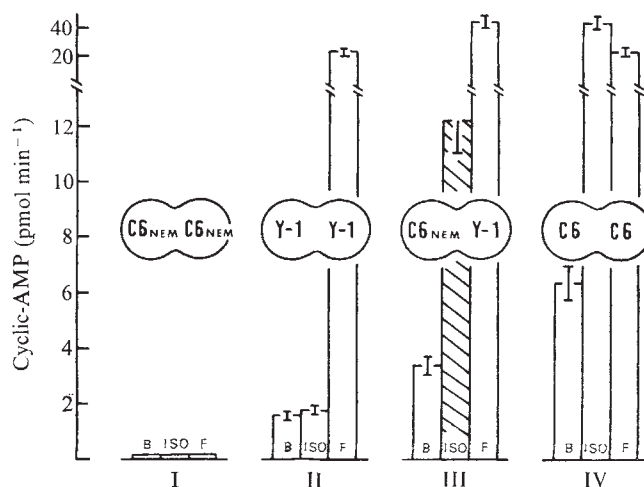


Fig. 3 Coupling of the β -adrenergic receptor of C6 cells with adenylate cyclase of Y-1 cells. Systems were: (i) $C6_{NEM}$, 1.2×10^7 fused with each other; (ii) Y-1 cells, 2.7×10^7 fused with each other; (iii) heterologous fusion between $C6_{NEM}$ cells, 1.2×10^7 and Y-1 cells, 2.7×10^6 ; (iv) untreated C6 cells, 1.2×10^7 fused with each other. The amount of virus was 400 HAU in systems i, ii, iv and 80 HAU in system iii. After 28 min at 37 °C the systems were placed on ice and cell ghosts were prepared. Other details are as in Fig. 1. Essentially the same results were obtained in a different experiment carried out in the presence of protein synthesis inhibitors: 15 min before fusion all cell suspensions received emetine, 20 μ M and cycloheximide, 20 μ M. The inhibitors remained present during fusion.

ferred from one adenylate cyclase system to another. Since the F cell showed strong activation of its adenylate cyclase by PGE_1 , transfer of the PGE_1 receptor was attempted. Figure 4a shows that the adenylate cyclase system of Y-1 cells can be efficiently coupled to the PGE_1 receptor of NEM-treated F cells. As a control, activation of adenylate cyclase by PGE_1 was also tested on Y-1 cells fused with C6 cells (system III of Fig. 3 served in the test). PGE_1 did not increase enzyme activity of the fused preparation. Therefore, the response cannot be due to unmasking of a receptor in Y-1 by fusion *per se* with a different cell. The PGE_1 receptor could also be contributed by F cells which had been heat treated to inactivate the adenylate cyclase (Fig. 4b). Thus such experiments are not exclusively dependent on the inactivation by NEM. Fusion of the heat treated cells with each other or with HTC cells did not reactivate the adenylate cyclase. The latter is a particularly important control since HTC cells are vigorously growing though they lack adenylate cyclase activity¹⁶. HTC cells also did not reactivate NEM-treated F cells or NEM-treated C6 cells which were used in experiments of this study. It is also of interest that the PGE_1 receptor and the β -adrenergic receptor⁷ are both more heat resistant than the adenylate cyclase to which

Table 1 Conservation of affinities of the β -adrenergic receptor after transfer to a heterologous adenylate cyclase system*

Cells in fusion	Apparent dissociation constants		
	1-Adrenaline μ M	1-Noradrenaline μ M	dl-propranolol nM
E	2.0	1.6	11
E_{NEM} -Y-1	1.8	1.1	9
E_{NEM} -Fc	1.5	1.0	—

*Fusion systems were prepared as described in legend of Fig. 1. Adenylate cyclase activities were determined on the cell ghosts in the presence of various hormone concentrations and the constants were calculated from a Lineweaver-Burk plot. Various amounts of 1-epinephrine were added to 0.1 μ M propranolol to determine the dissociation constant of the blocker.

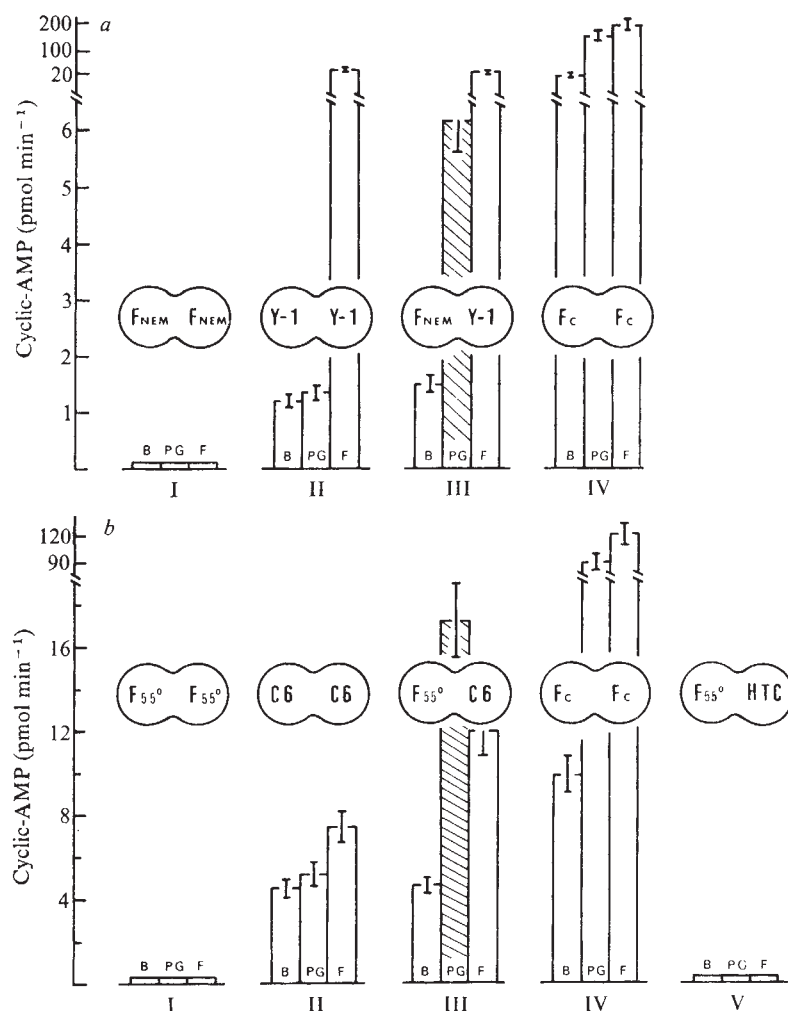


Fig. 4 Coupling of the prostaglandin E_1 receptor of F cells to adenylate cyclase of other cells. *a*, NEM-treated F cells (F_{NEM}) fused with Y-1 cells, systems were: (i) F_{NEM} , 1.3×10^7 fused with each other; (ii) Y-1 cells, 2.3×10^6 fused with each other; (iii) heterologous fusion between F_{NEM} cells, 1.3×10^7 and Y-1 cells, 2.3×10^6 ; (iv) untreated Fc cells, 1.3×10^7 fused with each other. Other details are as in Fig. 1, except that the hormone was prostaglandin E_1 (PG) $10 \mu M$ (given by Dr J. Pike, Upjohn Co., USA). The amount of virus was 400 HAU in systems i, iii, iv and 200 HAU in system ii. After 14 min incubation at $37^\circ C$ systems were placed on ice and cell ghosts were prepared by mild sonication¹⁷. Cells were sedimented by centrifugation for 3 min at 200g and the pellet was suspended in 0.35 ml of Na^+ salt medium containing 3 mM β -mercaptoethanol and 0.5 mg ml^{-1} STI. The cell suspension was sonicated for 15 s in a bath sonicator (Laboratory Supplies Co., Model T-80-80-1). *b*, Heat-treated F cells (F_{55}) fused with C6 cells systems were: (i) F_{55} , 1.2×10^7 fused with each other; (ii) C6 cells, 3×10^6 fused with each other; (iii) heterologous fusion between heated F cells, 1.2×10^7 and C6 cells, 3×10^6 ; (iv) F cells, 1.2×10^7 fused with each other; (v) heterologous fusion between heated F cells, 1.2×10^6 and HTC cells, 2.5×10^6 . HTC cells were grown in suspension¹⁹ and supplied by Dr R. Kulka of this department. F cells were suspended in 1.6 ml of Na^+ salt medium in a glass test tube to give 6×10^7 cells ml^{-1} . The cells were heated at $55^\circ C$ for 4 min and cooled in ice. Damage to the cells by the heat treatment was not visible by phase contrast microscopy. The amount of virus was 500 HAU in systems i, iii, iv, v and 100 HAU in system ii. After 15 min at $37^\circ C$ the fused cells were transferred to the cold and sonicated as described in Fig. 4a. Other details as in Fig. 1.

they were originally coupled. The PGE_1 receptor of NEM-treated F cells was also transferred to adenylate cyclase of C6 cells. PGE_1 increased enzyme activity 3.7-fold in the fused membranes (data not shown).

Experiments are in progress to transfer the ACTH receptor of the Y-1 cells to other cell types. Adenylate cyclase stimulation by ACTH in the strain of Y-1 cells used has, however, so far been rather weak and variable.

Experimental techniques

The coupling of a hormone receptor from one cell with the adenylate cyclase of another cell by the procedure described, is based on several essential requirements. Means and conditions for massive fusion must be provided because the transfer of receptor will only be evident if a considerable amount of the total adenylate cyclase in the system has been joined by the heterologous receptor. With cells having a high basal activity of adenylate cyclase, it may be difficult to demonstrate significant hormonal stimulation by coupling to a heterologous receptor. When using Sendai virus as a fusing agent the amount of virus must be accurately titrated. Both excess virus and prolonged incubation after fusion cause severe lysis of cells and inactivation of adenylate cyclase. The age and growth conditions of the cells used is also critical. For instance, adenylate cyclase in young cultures of C6 shows little response to catecholamine²⁰. F cells in the stationary phase were unsuitable for heat or NEM treatment since they underwent lysis during fusion or failed to fuse. In fact, it has been our routine observation that NEM- or heat-treated cells fuse less efficiently with each other as compared with their heterologous fusion with

native untreated cells. However, in turkey erythrocytes NEM treatment encouraged fusion. It is difficult to distinguish heterologous from homologous fusion when the partners are of similar appearance under the phase contrast microscope (C6, Fc and Y-1). Since NEM-treated cells fuse less readily with each other, counting the percentage of unfused cells serves as a rough indication of the extent of heterologous fusion.

Receptor-enzyme matching

It is obvious from our past⁷ and present data that matching of a receptor to a heterologous adenylate cyclase system readily occurs with a considerable variety of cells of different origin, type of differentiation and method of growth. As yet, we have not encountered a fusible cell type which failed to serve in heterologous receptor-enzyme matching. Blood cells, cells grown in suspension or cells grown in monolayer and subsequently suspended by trypsinisation served equally well. The fact that the cells already possessed one native receptor (PGE_1 in F cells, ACTH in Y-1 cells, β -adrenergic in C6 cells) did not prevent acceptance of an additional transferred receptor. The same cell could contribute the adenylate cyclase, in coupling with a foreign receptor, or contribute the receptor in coupling with a foreign adenylate cyclase (Fc-C6_{NEM}, F_{NEM}-C6). These observations considerably broaden our recent conclusion⁷ that the receptor is a unit independent of the enzyme and allows some suggestions as to the nature of the different hormone receptors. The unhindered transfer of two different receptors to adenylate cyclase systems of several cells suggests that the mechanism of coupling between receptors and adenylate cyclase may

be universal for all eukaryotic cells. The change that must occur in the receptor upon binding of the specific hormone, which results in activation of the enzyme, is probably also identical for all these hormone receptors. Thus, it is likely that all hormone receptors which couple to adenylate cyclase have a common chemical structure, differing only in the confined area which binds the specific hormone. Because of these considerations it is expected that when hormone receptors will become available as purified proteins, it will be possible to implant them in the adenylate cyclase systems of many eukaryotic cells.

More immediate application of the technique could be to determine why OS3 and Y-6 adrenal tumour cells fail to respond to ACTH with adenylate cyclase activation^{21,22} and to test for 'silent' receptors⁷ as apparently demonstrated for the β -adrenergic receptor of HTC cells. It would also be interesting to see whether the β -adrenergic receptor from a unicellular organism (such as *Tetrahymena*²³) is compatible with the adenylate cyclase of primates.

We thank Mrs N. Zakai and Dr A. Loyter for much advice about cell fusion and for supply of Sendai virus. The work was supported by grants from the National Institutes of Health, USA, and the U.S.-Israel Binational Science Foundation. M.S. is an established investigator of the Israel Ministry of Health.

Received 28 March; accepted 19 May 1977.

- ¹ Harris, H. *Nucleus and Cytoplasm* 3rd edn, 109-141 (Clarendon, Oxford, 1974).
- ² Okada, Y. *Expl Cell Res.* **26**, 98-103 (1962).
- ³ Ahkong, Q. F., Fisher, D., Tampion, W. & Lucy, J. A. *Biochem. J.* **136**, 147-155 (1973).
- ⁴ Loyter, A., Zakai, N. & Kulka, R. G. *J. Cell Biol.* **66**, 292-304 (1975).
- ⁵ Singer, S. J. *Cell Membranes* (eds Weissmann, G. & Clairborne, R.) 35-44 (H. P. Publishing Co. Inc., New York, 1975).
- ⁶ Frye, C. D. & Edidin, M. *J. Cell Sci.* **7**, 319-335 (1970).
- ⁷ Orly, J. & Schramm, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4410-4414 (1976).
- ⁸ Yasumura, Y., Buonassisi, V. & Sato, G. *Cancer Res.* **26**, Part 1, 520-535 (1966).
- ⁹ Salomon, Y., Londos, C. & Rodbell, M. *Analyt. Biochem.* **58**, 541-548 (1974).
- ¹⁰ Toister, Z. & Loyter, A. *Biochem. biophys. Res. Commun.* **41**, 1523-1530 (1970).
- ¹¹ Benda, P., Lightbody, J., Sato, G., Levine, L. & Sweat, W. *Science* **161**, 370 (1968).
- ¹² Amano, T., Hamprecht, B. & Kemper, W. *Expl Cell Res.* **85**, 399-408 (1974).
- ¹³ Pohl, S. L., Krans, H. M., Kozyreff, V., Birnbaumer, L. & Rodbell, M. *J. biol. Chem.* **246**, 4447-4454 (1971).
- ¹⁴ Schramm, M. *Adv. Cyclic Nucleotide Res.* **5** (eds Drummond, G. I., Greengard, P. & Robison, G. A.) 105-115 (Raven, New York, 1974).
- ¹⁵ Lands, A. M., Arnold, A., McAuliffe, J. P., Luduena, F. P. & Brown, T. G. *Nature* **214**, 597-598 (1967).
- ¹⁶ Granner, D., Chase, L. B., Aurbach, G. D. & Tomkins, G. M. *Science* **162**, 1018-1020 (1968).
- ¹⁷ Achar, S. B., Strada, S. J., Sander, R. B., Robison, G. A. & Thompson, W. J. *Fed. Proc.* **35**, 1203 (1976).
- ¹⁸ Insel, P. A., Maguire, M. E., Gilman, A. G., Bourne, H. R., Coffino, P. & Melmon, K. L. *Mol. Pharmacol.* **12**, 1062-1069 (1976).
- ¹⁹ Martin, D., Tomkins, G. M. & Granner, D. *Proc. natn. Acad. Sci. U.S.A.* **62**, 248-255 (1969).
- ²⁰ Schwartz, J. P., Morris, N. R. & Breckenridge, B. McL. *J. biol. Chem.* **248**, 2699-2704 (1973).
- ²¹ Schimmer, B. P. & Rae, P. A. *Adv. Cyclic Nucleotide Res.* **5** (eds Drummond, G. I., Greengard, P. & Robison, G. A.) 811 (Raven, New York, 1974).
- ²² Londos, C., Salomon, Y., Lin, M. C., Harwood, J. P., Schramm, M., Wolff, J. & Rodbell, M. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3087-3090 (1974).
- ²³ Kassis, S. & Kindler, S. H. *Biochim. biophys. Acta* **391**, 513-516 (1975).
- ²⁴ Racker, E. in *Dynamics of Energy Transducing Membranes* (eds Ernster, L., Eastabrook, R. W. & Slater, E. C.) 269-281 (Elsevier, Amsterdam, 1974).

Self-assembly and catalytic activity of the pyruvate dehydrogenase multienzyme complex of *Escherichia coli*

David L. Bates*, Michael J. Danson, Geoffrey Hale, Elizabeth A. Hooper & Richard N. Perham

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, UK

Studies of the self-assembly of pyruvate dehydrogenase of Escherichia coli show that the catalytic activity is proportional to the state of assembly and uncover a novel functional connection of active sites in this multienzyme complex.

The pyruvate dehydrogenase multienzyme complex of *Escherichia coli*^{1,2} catalyses the overall reaction



The complex, which is somewhat larger than a ribosome, is composed of multiple copies of three different types of polypeptide chain, responsible for the three constituent enzymic activities: pyruvate decarboxylase (E1) [EC 1.2.4.1], lipoate acetyltransferase (E2) [EC 2.3.1.12] and lipoamide dehydrogenase (E3) [EC 1.6.4.3]. The commonly accepted mechanism is outlined in Fig. 1.

The complex can be taken apart and the component enzymes reassembled *in vitro* to form the original structure³ and since enzymes E1 and E3 do not associate with each other in the absence of enzyme E2, it is clear that the latter forms the structural core of the assembled complex. It is probable that enzyme E2, like the corresponding lipoate succinyltransferase from the 2-oxoglutarate dehydrogenase complex, has octahedral

(432) symmetry⁴, which implies that it is composed of 24 polypeptide chains^{2,4}. These arguments from X-ray diffraction and electron microscopy^{2,5} apparently dispose of an alternative model in which E2 comprises 16 polypeptide chains⁶. On the other hand, two conflicting versions of the number of polypeptide chains in the intact complex are current. It has been proposed^{7,8} that there are about 24 chains of E1, 24 chains of E2 and about 12 chains of E3 whereas other measurements^{6,9} indicate that the complex generally carries a molar excess of E1 chains, the polypeptide chain stoichiometry (E1: E2: E3) having an upper limit of 2:1:1 (refs 9, 10).

Three active sites in three different component enzymes participate in the overall reaction (Fig. 1). The substrate is borne by a lipoic acid residue which is covalently linked through its carboxyl group to the N⁶-amino group of a lysine residue in each chain of the E2 component¹¹. The lipoyl-lysine 'swinging-arm' is postulated to rotate among the catalytic centres of the three enzymes^{3,12} and a study of the electron spin resonance spectra of spin-labelled lipoamide residues has provided the first convincing physicochemical evidence consistent with this hypothesis^{13,27}. But, modifications to the simple reaction mechanism presented in Fig. 1 must be envisaged since we now know that each E2 chain bears not one but two functionally active lipoyl residues¹⁴.

In this article we describe two new sets of experiments. We examine the self-assembly of the enzyme complex from the separated E1 component and E2-E3 subcomplex. We show that the chain ratio E1: E2 reaches a limiting value of 2:1 and that catalytic activity in the overall reaction is proportional to the state of assembly of the complex. We go on to examine the

* Present address: Department of Biochemistry, Stanford University Medical Center, Stanford, California 94305, USA.

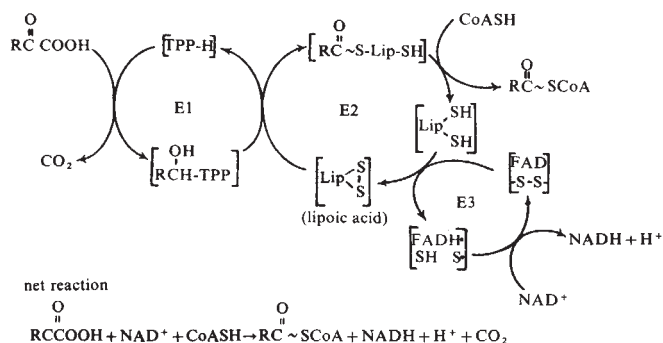


Fig. 1 The reaction mechanism of the pyruvate dehydrogenase multienzyme complex of *E. coli*. E1 (pyruvate decarboxylase, EC 1.2.4.1), E2 (lipoate acetyltransferase, EC 2.3.1.12) and E3 (lipoamide dehydrogenase, EC 1.6.4.3) represent the three enzymic activities that make up the complex. TPP-H, thiamin pyrophosphate.

fate of substrate within the complex and demonstrate participation of the lipoyl-lysine swinging-arms in subunit interactions of a novel and hitherto unsuspected kind.

Self-assembly and catalytic activity

Pyruvate dehydrogenase complex was prepared by the method of Reed and Mukherjee¹⁵ from a strain of *E. coli* K12 constitutive for the enzyme (provided by Professor H. L. Kornberg). The complex was dissociated into free E1 and E2-E3 subcomplex at pH 10 and these were resolved by gel filtration on a column of Sepharose 6B (refs 16, 17). The concentrations of the resultant stock solutions were determined by amino acid analysis¹⁸. The polypeptide chain stoichiometry (E1 : E2 : E3) of the E2-E3 subcomplex was found to be 0.06 : 1.0 : 0.88 by means of the radio-amidation method of Bates *et al.*⁹. The E1 component gave the single band expected on sodium dodecylsulphate (SDS)-polyacrylamide gel electrophoresis. A series of partly reassembled complexes was then prepared by adding increasing amounts of E1 to a constant amount (0.29 mg) of E2-E3 subcomplex in 50 mM sodium phosphate buffer, pH 7.0. Each mixture was held for at least 10 h at 0 °C and any unbound E1 was then separated from assembled complex by gel filtration on Sepharose 6B. Initially, all the E1 added became bound and a single high molecular weight species emerged from

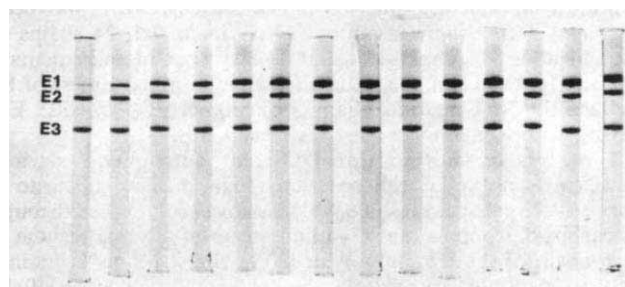


Fig. 2 Analysis by SDS-polyacrylamide gel electrophoresis of the self-assembly of the pyruvate dehydrogenase multienzyme complex. A series of partly reassembled complexes was prepared by adding increasing amounts of E1 to a constant amount (0.29 mg) of E2-E3 subcomplex in 50 mM sodium phosphate buffer, pH 7.0, containing 2 mM EDTA and 1 mM sodium azide. The protein concentrations were determined by amino acid analysis. After 10 h at 0 °C, the mixtures were gel filtered on a column (650 × 6.5 mm) of Sepharose 6B in the same buffer, and the fractions containing the complex were pooled. Samples were submitted to SDS-polyacrylamide gel electrophoresis in standard conditions¹⁹ and the gels correspond, from left to right, with the points plotted in Fig. 3. The subunit molecular weights are: E1, 100,000; E2, 80,000 and E3, 56,000 (refs 6, 19).

the Sepharose column. But, when the amount of E1 in the assembly mixture reached 0.41 mg, free E1 component began to appear in the low molecular weight region of the column eluate and thereafter any additional E1 was found in this peak. As judged by SDS-polyacrylamide gel electrophoresis and by enzyme assay¹⁴ of the column eluates, no E3 was displaced from the complex during the assembly.

Analysis of the series of partly reassembled complexes by SDS-polyacrylamide gel electrophoresis is shown in Fig. 2. An upper limit to the binding of E1 to the E2-E3 subcomplex is evidently very tight. The subcomplex is apparently saturated when 1.8 chains of E1 are bound per chain of E2, as measured by the amidation assay of the assembled complexes. This result is supported by the observation (Fig. 3) that complete assembly and activity were only reached when the molar ratio of E1 : E2 chains (the latter as subcomplex) in the assembly mixture was 2 : 1, as measured by amino acid analysis. We estimate that the binding constant for E1 to the E2-E3 subcomplex must be in excess of 10^7 M^{-1} to account for the shape of the curve in Fig. 3. The E3 content of the series was constant at a mean value of 0.85 chains per chain of E2, which does not differ significantly from the value of 0.88 in the starting subcomplex. This experiment fully supports our suggestions that the limiting polypeptide chain stoichiometry is 2 : 1 : 1 (refs 9, 10).

The E3 activity of the complex (that is, the reduction of NAD^+ by exogenous dihydrolipoamide) is unaffected by the binding of E1, as was shown by assaying a sample of E2-E3 subcomplex for this activity in the presence of increasing amounts of E1. Since the E2:E3 chain ratio for the series of reassembled complexes was constant, we could therefore use the E3 activity as a measure of the amount of subcomplex in each species. Thus we could determine how the overall pyruvate dehydrogenase activity varied with the E1 content of the complex. Accordingly, the overall complex and E3 activities of the partly assembled enzyme complexes were measured using standard assays¹⁴ and the ratios of these activities are also plotted in Fig. 3. The increase in overall complex activity closely followed the binding of E1, maximum activity being reached only when the subcomplex had bound its full complement of E1 chains. The effective specific activity of the E1 component in the complex was therefore at least approximately constant.

Reaction mechanism and the interaction of active sites

We had previously shown that each E2 chain bears two functionally active lipoic acid residues¹⁴. We have now demonstrated that the maximal overall complex activity is achieved when the limiting chain stoichiometry (E1 : E2) of 2 : 1 is reached (Fig. 3). The free E1 component is thought to be a dimer^{7,20} although it seems to dissociate at high pH into monomers^{10,21}. Only the dimer is catalytically active²¹. The simplest formulation of the generally accepted mechanism (Fig. 1) would envisage direct transfer of substrate through a fixed geometric arrangement of three active sites. This proposition was tested as follows.

In the presence of the substrate 2-¹⁴C-pyruvate, and the absence of CoA, the lipoic acid residues of the complex become acetylated and reaction then ceases (Fig. 1). The extent of acetylation can be measured by counting the radioactivity in samples of the complex after precipitation with trichloroacetic acid and this can then be correlated with the E1 content of the complex. A new series of partly reassembled complexes was

therefore prepared as described above, except that the proportions of E1 and E2-E3 subcomplex in the assembly mixture were chosen to yield some complexes with lower E1 : E2 ratios. The ratios were then measured by the radio-amidination method⁹. Samples (each containing about 15 µg of E2-E3 subcomplex) of the partly reassembled complexes were incubated at pH 7.0 with 0.2 mM 2-¹⁴C-pyruvate in the absence of CoA and, after 2 min, the protein was precipitated with trichloroacetic acid and counted for radioactivity. The radioactivity incorporated was plotted against the E1 : E2 chain ratio (Fig. 4): as in the previous experiment, the measured E2 : E3 chain ratio and the E3 enzymic activity were used to normalise the results to the same constant amount of E2. The maximum extent of acetylation was calculated from the specific radioactivity of the 2-¹⁴C-pyruvate to be approximately two

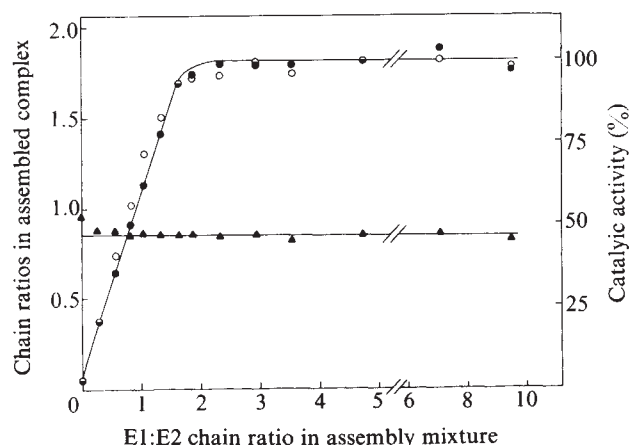


Fig. 3 The polypeptide chain stoichiometries and catalytic activities of the series of partly reassembled complexes described in Fig. 2. The polypeptide chain ratios, measured by radio-amidination⁹, are based on E2 as unity. These ratios (E1, ●; E3, ▲) are plotted against the chain ratios of E1 : E2 (the latter as subcomplex) in the assembly mixtures before gel filtration, as determined by amino acid analysis of the stock solutions. Five SDS gels were run for each sample and the maximum standard deviation encountered was 6%. A least-squares analysis of the first seven points gave a gradient of 0.99 and we estimate a minimum equilibrium constant of 10^7 M^{-1} for the binding of E1 to the E2-E3 subcomplex. The ratio of the overall complex activity¹⁴ to that of the E3 component¹⁴ for each sample is also plotted (○). The final value reached was 0.66 (compared with 0.7 in the complex before resolution) and the maximum overall complex activity recovered was 25 IU per mg, the same as the complex before resolution.

acetyl groups bound per E2 chain, in good agreement with our previous measurements of the lipoid content of the complex by other means¹⁴. It is clear from Fig. 4 that the extent of acetylation of lipoid residues on the E2 chains rose initially much more steeply than the E1 : E2 chain ratios of the complexes, implying that more than one E2 chain was 'serviced' by a given E1-dimer in the complex. A simple 1 : 1 correspondence of E1 chains bound with lipoid acid residues acetylated is obviously excluded.

The binding of E1 to the E2-E3 subcomplex is non-cooperative and the binding sites can be assumed to be identical (ref. 10 and unpublished work of W. Busch and U. Henning, quoted in ref. 25). Let us suppose that n E2-chains can be serviced by a single E1-dimer and conversely, therefore, that a given E2-chain can be acetylated by the action of any of n different E1-dimers. Let p be the ratio of E1 dimers to E2 polypeptide chains in a given complex. Then the probability that an E1 binding site is occupied is also p . If there is one binding site for an E1-dimer on each E2 chain, which is implied by the maximum polypeptide stoichiometry of 2 : 1, then the probability that an E2 chain will not be acetylated is the same

as the probability that n E1-dimers will not be bound, which is $(1-p)^n$. Thus the probability, f , that an E2 chain will be acetylated is

$$f = 1 - (1-p)^n \quad (1)$$

In the experiment, we have measured f as a function of the E1 : E2 polypeptide chain ratio, r . But $r = 2p$, so

$$f = 1 - (1-r/2)^n \quad (2)$$

This equation describes the curves which have been plotted for various integral values of n in Fig. 4. The experimental points lie closest to the line described by $n = 12$.

Rearrangement of equation (2) gives

$$\log(1-f) = n \log(1-r/2) \quad (3)$$

Replotting the experimental data from Fig. 4 in this form gives a good straight line of gradient, $n = 12 \pm 1$.

It should be noted that the form of the curves in Fig. 4 does not depend on the assumption that E1 is active only as dimers. Identical theoretical lines are obtained if random binding of active monomers to two sites on E2 is taking place. The value of n corresponding to any curve would then be halved since it would refer to the number of E2 chains whose acetylation is caused by a monomer of E1.

In the 'servicing' experiments we have described above, the enzyme complexes were incubated with 2-¹⁴C-pyruvate for 2 min before being precipitated with trichloroacetic acid. But, the same extent of acetylation was observed when the incubation time was reduced to 20 s.

Symmetry and transacetylation within the complex

The most plausible model for the structure of the E2 core of the multienzyme complex takes the form of a cube with

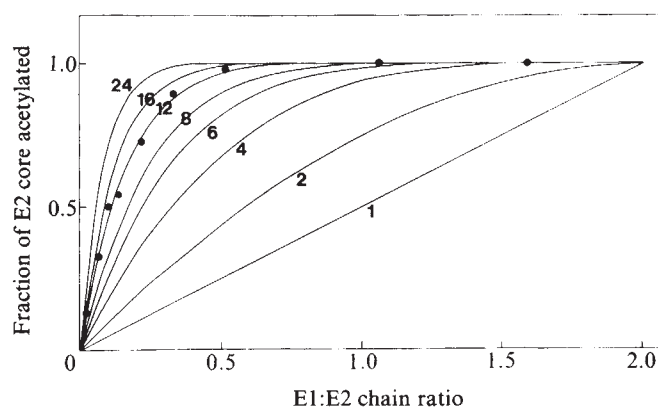


Fig. 4 Acetylation with 2-¹⁴C-pyruvate of the E2 component of a series of partly reassembled pyruvate dehydrogenase multienzyme complexes. Samples of pyruvate dehydrogenase complex were incubated at 0 °C with 0.2 mM 2-¹⁴C-pyruvate in 0.1 ml of 50 mM sodium phosphate buffer, pH 7.0, containing 0.7 mM thiamin pyrophosphate, 10 mM MgCl₂ and 1 mM NAD⁺. After 2 min, 50 µl of rabbit immunoglobulin G (Mann Research: 20 mg ml⁻¹ in 20 mM sodium phosphate buffer, pH 7.0) was added as carrier protein, followed immediately by 1 ml of ice-cold 12.5% trichloroacetic acid. The precipitated proteins were collected on glass microfibre filters (Whatman GF/C) and were washed with 25 ml of 12.5% trichloroacetic acid, 6 ml of acetone containing 50 mM HCl and finally 6 ml of diethyl ether. The dry filters were placed in vials with 4 ml of toluene containing 2,5-diphenyloxazole (5 g l⁻¹) and counted for radioactivity in a Nuclear Chicago Unilux II-A scintillation counter²². The stoichiometries of the polypeptide chains in the pyruvate dehydrogenase complexes were determined by radio-amidination⁹. The theoretical curves are plotted from the equation $f = 1 - (1-r/2)^n$, for integral values of n as shown, where f = fraction of the E2 core acetylated, r = chain ratio of E1:E2 and n = number of E2 chains acetylated by the action of one E1-dimer.

octahedral symmetry, comprising 24 polypeptide chains^{2,4}. It is clear from our experiments that n does not have the minimum value of 1 nor the potential maximum value of 24. One E1 dimer is not able to cause the acetylation of the whole E2 core. It would be expected *a priori*, however, that the observed value of n would be a multiple of one or more of the symmetry elements. For the octahedral model, n could have the values 1, 2, 3, 4, 6, 8, 12 or 24. Our present value of 12 can therefore be reconciled in principle with octahedral symmetry.

The possibility that one E1 dimer can interact direct with all 12 susceptible E2 chains simultaneously is discounted in view of the very large distances that would have to be spanned between the active sites (the complex is at least 30 nm in diameter^{1,2,23}), compared with the length (1.4 nm) of the lipoyl-lysine swinging arm. Similarly, rapid migration of the E1 dimer from one binding site to another can be ruled out since an answer of $n = 24$ would be expected if the binding sites remained equivalent. We are left with the possibility that acetyl groups can be transferred between lipoyl residues bound to neighbouring E2 chains in the core, a reaction we shall term 'transacetylation' and which should be distinguished from the transfer of acetyl groups to CoA to form product (Fig. 1). The diameter of a globular E2 polypeptide chain (molecular weight 80,000; refs 19, 24) would be of the order of 6 nm, and we know that there are two lipoyl-lysine swinging arms of length 1.4 nm each per E2 chain¹⁴, giving a potential total span of at least 5 nm for each subunit. Conformational changes in the complex might also facilitate transacetylation, so this mechanism seems structurally feasible.

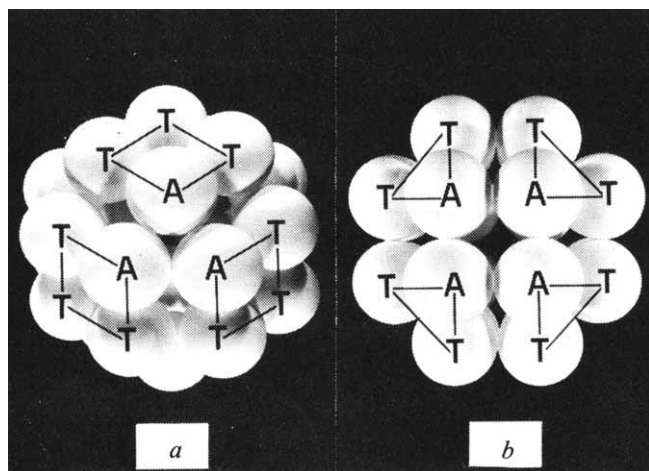


Fig. 5 Two models for the mechanism of acetylation of 12 E2 chains by means of a single E1-dimer in the pyruvate dehydrogenase multienzyme complex. The views are along (a), threefold and (b), fourfold axes of the E2 core component. The subunits acetylated by direct physical interaction with a single E1-dimer are marked with an A. Those acetylated by subsequent transacetylation are marked with a T and shown linked to the appropriate subunit of type A. The total number of chains acetylated in either model is 12.

If the postulated transacetylation reactions could take place between each and every subunit in the E2 core, n would obviously have the value 24. To limit it to 12, we must therefore restrict the transacetylation in some way. One possible model of the E2 core with octahedral symmetry is shown in Fig. 5. Every E2 chain can be regarded as one of the three subunits in a trimer cluster at each of the eight corners of the cube or as one of the four subunits arranged about the fourfold axis on each of the six faces of the cube. We propose two possible reaction schemes. Consider a single E1 dimer bound to any one E2 chain. This E1 dimer might cause the independent acetylation of all three E2 chains in the trimer cluster at a corner of the

cube by direct reaction, which would be followed by transacetylation among the four chains on each of the three cognate faces of the cube (Fig. 5a). Alternatively, the E1 dimer might cause the independent acetylation of four E2 chains by direct reaction with each of the subunits on a fourfold axis on the face of the cube, which would be followed by transacetylation among the three chains in the trimer clusters at each of the four corners of that face (Fig. 5b). We cannot for the moment choose between these two schemes but in both we must postulate that the subunits that become acetylated by direct reaction with the E1 dimer cannot engage in rapid transacetylation reactions among themselves.

Conclusions

Our results on the stoichiometry of polypeptide chains in the self-assembly of the complex substantiate our previous work^{9,10} and are in good agreement with the chain ratios of the native complexes isolated by Vogel *et al.*⁶ except that the highest value they recorded for the E1 : E2 chain ratio was 1.4. From our measurements of subunit stoichiometries and assuming octahedral symmetry for the E2 component, we would predict a molecular weight of about 8×10^6 for the fully assembled complex. This molecular weight predicted from symmetry arguments and protein chemistry has since been found to be in very good agreement with that measured independently by ultracentrifugation methods (M.J.D. and A. J. Rowe, unpublished).

The discovery of extensive internal transacetylation reactions in the complex has revealed a novel system of functional connection of active sites. The value of $n = 12$ gives a good fit of theory to experiment (Fig. 4) and, even if we are wrong in detail, n must clearly be very much greater than 1, the value implicit in the usual formulation of the mechanism (Fig. 1). Quenched-stopped-flow experiments²⁶ suggest that the transacetylation reactions are sufficiently fast to be kinetically competent in the normal enzymic reaction (M.J.D., A. R. Fersht and R.N.P., unpublished). They form a way of 'posting' acetyl groups and associated reduction of lipoyl residues from an E1 site to a physically distant E3 site. What advantage, if any, they confer may become apparent when the reaction is dissected in further detail.

We are grateful to the SRC, ICI and the Wellcome Trust for financial support. We thank Dr K. Sargeant and Mr A. R. Whitaker, Microbiological Research Establishment, Porton Down, for growing the bacteria.

Received 15 February; accepted 30 May 1977.

- 1 Reed, L. J. & Oliver, R. M. *Brookhaven Symp. Biol.* **21**, 397-412 (1968).
- 2 Reed, L. J. *Acc. chem. Res.* **7**, 40-46 (1974).
- 3 Koike, M., Reed, L. J. & Carroll, W. R. *J. biol. Chem.* **238**, 30-39 (1963).
- 4 DeRosier, D. J. & Oliver, R. M. *Cold Spring Harb. Symp. quant. Biol.* **36**, 199-203 (1972).
- 5 Perham, R. N. *Phil. Trans. R. Soc. B272*, 123-136 (1975).
- 6 Vogel, O., Hoehn, B. & Henning, U. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1615-1619 (1972).
- 7 Eley, M. H., Namihara, G., Hamilton, K., Munk, P. & Reed, L. J. *Archs Biochem. Biophys.* **152**, 655-669 (1972).
- 8 Reed, L. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 3068-3072 (1975).
- 9 Bates, D. L., Harrison, R. A. & Perham, R. N. *FEBS Lett.* **60**, 427-430 (1975).
- 10 Perham, R. N. & Hooper, E. A. *FEBS Lett.* **73**, 137-140 (1977).
- 11 Nawa, H., Brady, W. T., Koike, M. & Reed, L. J. *J. Am. chem. Soc.* **82**, 896-903 (1960).
- 12 Green, D. E. & Oda, T. *J. Biochem. (Tokyo)* **49**, 742-757 (1961).
- 13 Ambrose, M. C. & Perham, R. N. *Biochem. J.* **155**, 429-432 (1976).
- 14 Danson, M. J. & Perham, R. N. *Biochem. J.* **159**, 677-682 (1976).
- 15 Reed, L. J. & Mukherjee, B. *B. Meth. Enzym.* **13**, 55-61 (1969).
- 16 Reed, L. J. & Willms, C. R. *Meth. Enzym.* **9**, 247-265 (1966).
- 17 Coggins, J. R., Hooper, E. A. & Perham, R. N. *Biochemistry* **15**, 2527-2533 (1976).
- 18 Harrison, R. A. thesis, Univ. Cambridge (1974).
- 19 Perham, R. N. & Thomas, J. O. *FEBS Lett.* **15**, 8-12 (1971).
- 20 Vogel, O. & Henning, U. *Eur. J. Biochem.* **18**, 103-115 (1971).
- 21 Dennert, G. & Eaker, D. *FEBS Lett.* **6**, 257-261 (1970).
- 22 Brown, J. P. & Perham, R. N. *Biochem. J.* **155**, 419-427 (1976).
- 23 Durchschlag, H. *Biophys. Struct. Mechanism* **1**, 153-168 (1975).
- 24 Vogel, O., Beikirch, H., Müller, H. & Henning, U. *Eur. J. Biochem.* **20**, 169-178 (1971).
- 25 Henning, U., Vogel, O., Busch, W. & Flataard, J. E. in *Protein-Protein Interactions* (eds Jaenicke, R. & Helmreich, E.) 343-361 (Springer, Berlin, 1972).
- 26 Fersht, A. R. & Jakes, R. *Biochemistry* **14**, 3350-3356 (1975).
- 27 Grande, H. J., Van Telgen, H. J. & Veeger, C. *Eur. J. Biochem.* **71**, 509-518 (1976).

letters to nature

NP0532 and a hole in the Crab Nebula

THE Crab Nebula may account for as much as 35% of the dispersion measure, D , of its pulsar, NP0532 (ref. 1). Davidson and Terzian¹ have pointed out that if this is so, and if the ionised material is distributed uniformly over the volume of the nebula, then the Crab's expansion² of $\sim 1,000 \text{ km s}^{-1}$ should cause a decrease in D of about 0.07% per year. Isaacman and Rankin³ studied the dispersion measure over a 5-yr period, however, and observed no such decrease. From this, an upper limit to the Crab's contribution to D can be derived. It is much smaller than previously suspected.

The variability of D is illustrated in Fig. 1; there is no secular decrease and there is a 2-yr long linear rise beginning in mid-1972. The fractional increase, to mid-1974, is about 0.1%. The times of elevated dispersion measure—from August 1969 through May 1971, and from July 1972 onward—probably occur when the line of sight is interrupted by a turbulence cell of enhanced electron density in either the Crab Nebula or the interstellar medium. The former is more likely since no other pulsars exhibit marked dispersion changes. Thus the plateau regions of D —from mid-May 1969 to mid-July 1969 and from June 1971 through August 1972—probably represent the base contribution of the nebula plus the interstellar medium. The dilution of electrons in the nebula should appear as a negative slope in the plateau. Its absence allows us to place an upper limit on the Crab's contribution to D , provided that we can rule out any mechanism of increasing D that might cancel the dilution effect.

As a model, we assume that most of the contribution, D_{neb} arises from a thin spherical ionised shell of radius r and thickness Δ , rather than from a uniform sphere of electrons. We justify this on the basis of Shklovskii's observation⁴ that the filaments, the highest-density regions, lie on the periphery of the nebula. (Trimble⁵ has disputed this on the basis of radial velocity measurements.) Furthermore, radiation pressure will

tend to sweep out the interior of a shell, at least in the beam of the pulsar.

The dispersion measure is defined as $D = \int n_e dl$, the electron number density integrated along the line of sight. Thus, if N electrons are uniformly distributed over the volume V of the shell

$$D_{\text{neb}} = \frac{N\Delta}{V} = N/(4\pi r^2) \quad (1)$$

Therefore the time derivative is

$$\dot{D}_{\text{neb}} = \frac{-N}{4\pi r^2} 2 \frac{\dot{r}}{r} = -2 D_{\text{neb}}/T \quad (2)$$

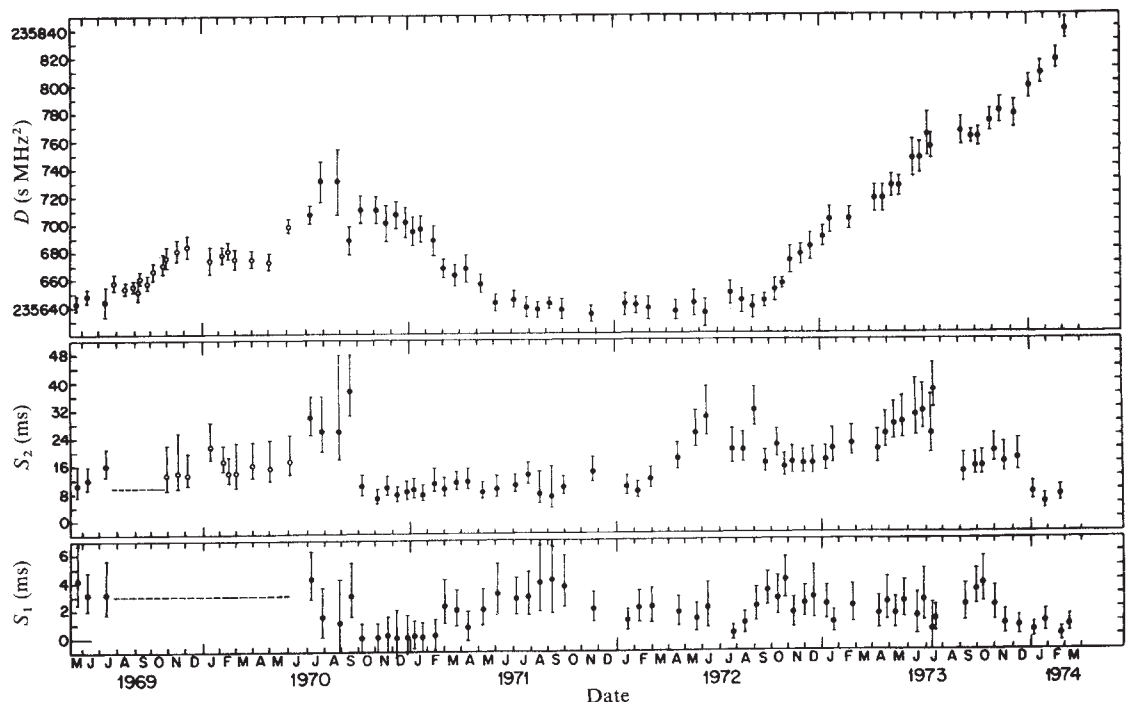
$T = r/\dot{r}$ is the 'kinetic age' of the nebula, 830 yr². From the two plateaus in Fig. 1, we can place an upper limit of 5 s MHz² yr⁻¹ on the decline of D (1 s MHz² = 7.43×10^{14} electrons cm⁻² = 2.41×10^{-4} pc cm⁻³), from which it follows that $D_{\text{neb}} < 2,100$ s MHz². This is slightly less than 1% of the total dispersion measure, 235,650 s MHz² (56.8 pc cm⁻³). The result can be written as $\Delta = 0.5/n_e$ where Δ is measured in pc and n_e in cm⁻³. For n_e comparable with filamentary densities ($\sim 10^3 \text{ cm}^{-3}$), the shell must be paper-thin compared with the nebula. It seems more likely that we observe the pulsar through a hole in the nebula, possibly cleaned out by the pulsar, where $n_e \sim 10$. It can now be seen that the assumption of a spherical shell was not crucial: a filled volume would require an even lower value of n_e to keep D_{neb} within the observed limit.

This determination of the Crab's fraction of the dispersion puts a constraint on the mass of ionised material it contains. For a spherical shell, the mass is

$$M = 4\pi r^2 n_e m_p (1+4f)\Delta \quad (3)$$

where m_p is the mass of the proton and f is the fractional abundance of He nuclei. Taking $f = 0.1$, $r = 1$ pc, and $n_e \Delta = D_{\text{neb}} \leq 0.5 \text{ pc cm}^{-3}$ gives $M = 0.21 M_\odot$. This must be

Fig. 1 Variability of the dispersion measure, D , of NP0532. (1 s MHz² = 7.43×10^{14} electrons cm⁻².) S_1 and S_2 are pulse-smearing time constants for a two-thin-screen multipath scattering model.



regarded as a lower limit, since looking through an abnormally sparse part of the nebula makes the total mass appear deceptively low. Trimble and Woltjer⁵ derive $M \sim 1M_{\odot}$ on dynamical grounds.

Since the contribution of the interstellar medium to the dispersion measure is higher than previously thought, the average electron density $\langle n_e \rangle$ in the line of sight (excluding the nebula) must be revised upwards. Davidson and Terzian derived $\langle n_e \rangle = 0.019 \text{ cm}^{-3}$ based on a 2-kpc distant nebula and $D_{\text{neb}} = 1/3 D$. Using a more accurate figure for the distance² (1.7 kpc) and $D_{\text{neb}} = 0.01 D$ gives $\langle n_e \rangle = 0.033 \text{ cm}^{-3}$.

It is important that all of this holds true only if $D = D_{\text{neb}}$, that any slope in the plateau of D in Fig. 1 is due entirely to nebular expansion. In principle, D_{neb} could be declining at the rate called for by Davidson and Terzian while being exactly compensated for by an increase in electron density elsewhere along the line of sight. In particular, those authors have pointed out that the Strömgren sphere engendered by ultraviolet and X-ray emission from the nebula has not had time to come to equilibrium. Integrating the Crab's spectrum⁶ above the Lyman limit (by smoothly connecting the optical and X-ray spectra with a curve of spectral index 1.1) shows that the nebula puts out approximately as much ionising radiation as an O8 type star. If the density of atoms in the interstellar medium is $n_{\text{ism}} = 0.15 \text{ cm}^{-3}$, a Strömgren sphere of radius 70 pc will form in $5 \times 10^6 \text{ yr}$. Since the nebula is less than 10^3 yr old, the ionisation front should be expanding, causing the dispersion measure of the pulsar to increase. Mitigating this effect is the extant Strömgren sphere of unknown size formed by the pre-supernova object. The contribution to D will change at the rate $D_{\text{ss}} = n_{\text{ism}} R$, where R is the velocity of the ionisation front. Provided $R \leq 750 \text{ km s}^{-1}$, we have $D_{\text{ss}} \leq 0.5 \text{ MHz}^2 \text{ yr}^{-1}$, which is insignificant. Since the expected ionisation front velocity a few tens of parsecs from the nebula is only a few hundred km s^{-1} (ref. 7), this will be the case. Hence this mechanism cannot compensate for the expansion of the nebula.

Another possible source of variation in D , which can, in principle, give rise to a systematic increase, is the 'wisps' near the pulsar, identified by Scargle⁸. These lie $\sim 0.01 \text{ pc}$ from the pulsar, and from their motions it seems that they are hydro-magnetically coupled to it. However, their low surface brightness and the lack of Faraday depolarisation of the pulsar radiation implies that their density is probably very low. Since their characteristic size is $\sim 0.01 \text{ pc}$, they contribute negligibly to the pulsar's dispersion measure.

Since the pulsar and the nebula are the only sources of ionising photons, any other mechanism for causing a secular increase in D must invoke the appropriate density fluctuations in the line of sight. The motions of these regions would have to compensate very precisely for the rate of dilution of electrons in the nebula, so their existence is improbable. We conclude that the dispersion plateau does indeed represent the base contribution of the Crab Nebula (in the line of sight to the pulsar) to the interstellar medium, from which it follows that (1) the nebula contributes less than 1% of the total dispersion measure of NP0532, (2) we are looking at the pulsar through a low-density hole in the nebula, (3) the mass of the nebula is at least $0.2M_{\odot}$, and (4) the average electron density of the interstellar medium in the direction of the Crab is 0.033 cm^{-3} .

I thank Professors Yervant Terzian and John Rankin for several useful discussions on the subject. This work was supported by the National Astronomy and Ionosphere Center, which is operated by Cornell University under contract to the National Science Foundation.

R. ISAACMAN

*Huygens Lab, Sterrewacht,
Leiden 2405,
The Netherlands*

Received 28 February; accepted 16 May 1977.

¹ Davidson, K. & Terzian, Y. *Astronom. J.* **74**, 849–854 (1969).

² Trimble, V. *Astronom. J.* **73**, 535–547 (1968).

³ Isaacman, R. & Rankin, J. *Astrophys. J.* **214**, 214–232 (1977).

⁴ Shklovskii, I. S. *Supernovae* (Wiley, London, 1968).

⁵ Trimble, V. & Woltjer, L. *Astrophys. J.* **163**, L97–L98 (1971).

⁶ Baldwin, J. E. *Proc. IAU Symposium* **46**, 22–31 (1970).

⁷ Spitzer, Jr., L. *Diffuse Matter in Space* **184** (Wiley, London, 1968).

⁸ Scargle, J. *Astrophys. J.* **156**, 401–424 (1969).

The optical variability of 3C345

A SURVEY of the optical variability of compact extragalactic objects has been in progress at Asiago Observatory since 1967. Observations are taken mainly with the 67/92/215 cm Schmidt telescope on Eastman 103a-O emulsion plus a 2-mm GG 13 filter to match the B-system. A full account of the data obtained so far is now in preparation and will be published elsewhere, but it is worthwhile to report immediately on the variability of the quasar 3C345 because of its remarkable characteristics. Since Kinman *et al.*¹ discovered the large optical variations and the evidence of periodicities of 80 and 320 d, several papers have appeared reporting photometric observations of this object, whose behaviour is, therefore, unusually well covered. Also, 3C345 displays an apparent 'super-light' expansion at radio frequencies (see Cohen *et al.*²).

We have collected in Fig. 1, 485 B-magnitudes by Kinman *et al.*¹, Barbieri and Erculiani³, Tritton and Selmes⁴, Smyth and Wolstencroft⁵, McGimsey *et al.*⁶, Selmes *et al.*⁷, Markova *et al.*⁸, Markova and Zhukov⁹ and from unpublished data obtained at Asiago. To simplify discussion, observations in other photometric systems (such as P-magnitudes) have been discarded and values obtained on the same day have been averaged together, so that the minimum time interval between two points in Fig. 1 is one day. The timespan covered by the data is 3,965 d. Three strong brightenings can be recognised during this period, when the object reached magnitudes brighter than the 16th. In the first and third occasion (1967 and 1971 respectively), the behaviour was very complex, with oscillations larger than 0.5 mag taking place in less than 10 d; on the second occasion (mid-1969) there was a single burst of short duration not accompanied by the slow increase seen before and after. To analyse this light curve in a quantitative way, we have employed a procedure suggested by Deeming¹⁰ for the Fourier analysis of unequally spaced data.

A fuller discussion of our data are given elsewhere¹¹; here only an important result is reported. The power spectrum PN shows three peaks, a narrow one at $\nu = 0.00065$ (period 1,540 d), a broader one at $\nu = 0.00125$ (period 800 d), and finally a smaller peak at 140 d. The region around 80 d shows an irregular structure with no definite feature, and actually the disappearance of this periodicity after a few cycles has already been discussed by Kinman¹². The precise value of the largest period is not well determined by the timespan of the data in Fig. 1, 3,965 d, and, therefore, periods ranging from 1,420 to 1,650 d can be considered equally possible. One of them, however, is *a priori* especially attractive, that at 1,600 days, not only because it is a multiple of 800 but also because its difference of phase with the component at 800 d is almost exactly $\pi/2$ (the phases are computed from the imaginary and real parts of the Fourier transform). We have therefore attempted to synthesise the data of Fig. 1 with these three harmonics. Regarding their amplitudes, in the present situation the total lack of data when the object is close to the Sun and the scanty information available on other occasions will appreciably reduce PN at periods lower than one year. After trials, the amplitude of the component at 140 d has been increased to 0.25 mag from its computed value of 0.14 mag. The other two harmonics have amplitudes equal to $(2PN)^{1/2}$.

The solution is then

$$f(t) = 16.61 + 0.35 \cos(2\pi t/1600 + 0.85) \\ + 0.32 \cos(2\pi t/800 - 0.72) + 0.25 \cos(2\pi t/140 + 0.46)$$

which is shown in Fig. 1 as the solid line. This solution, reproduces with fair accuracy the mentioned complex maxima and single burst, although it is clearly impossible to have a precise

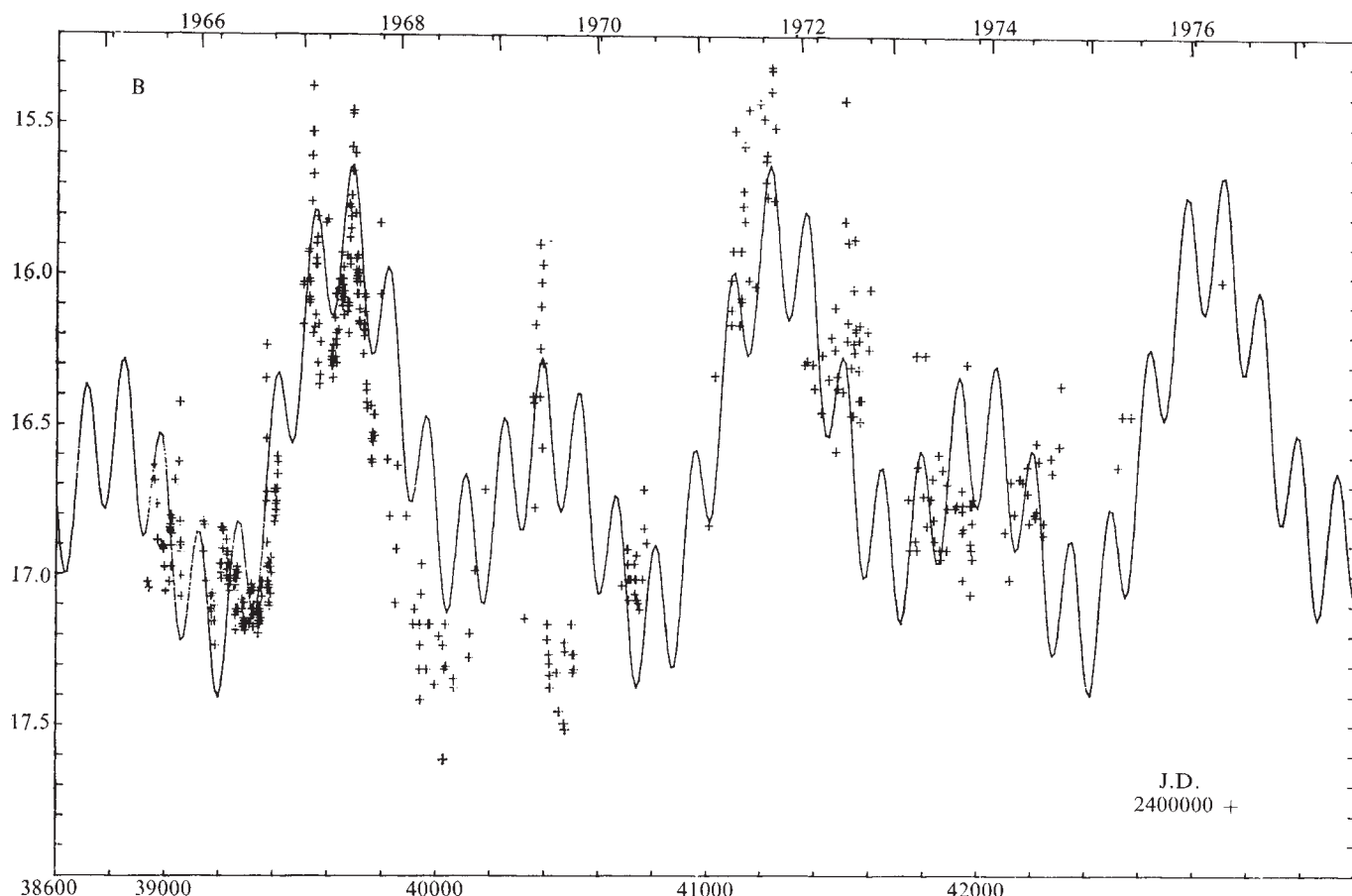


Fig. 1 The light curve of 3C345 from 1965 to 1976. +, B magnitudes taken from the literature quoted in the text. —, Harmonic solution $f(t)$ derived from our analysis.

representation of this latter feature with low-frequency components.

Other solutions with periods slightly different from 1,600 and 800 d have been explored and obviously give a rather similar representation. The best periods can be obtained only by a long extension of the data in the future or in the past. Historical data on the light curve of 3C345 have been published by Angione¹³ and cover the years from 1896 to 1953. If the solution of Fig. 1 is extended over these past observations, a good agreement is found although, unfortunately, the points are too few to be conclusive evidence. Observations in the years to come will be necessary to prove beyond doubt the presence of periodic phenomena in 3C345.

C. BARBIERI
G. ROMANO
S. DI SEREGO
M. ZAMBON

Istituto di Astronomia,
Università di Padova,
I-35100 Padova, Italy

Received 10 May; accepted 1 June 1977.

- ¹ Kinman, T. D., Lamla, E., Ciurla, T., Harlan, E. & Wirtanen, C. A. *Astrophys. J.* **152**, 357–374 (1968).
- ² Cohen, M. H., Moffet, A. T., Romney, J. D., Schilizzi, R. T., Seielstad, G. A., Kellermann, K. J., Purcell, G. H., Shaffer, D. B., Pauliny-Toth, I. I. K., Preuss, E., Witzel, A. & Rinehart, R. *Astrophys. J.* **206**, L1–L3 (1976).
- ³ Barbieri, C. & Abati-Erculiani, L. *Mem. S.A.It.* **39**, 421–428 (1968).
- ⁴ Tritton, K. P. & Selmes, R. A. *Mon. Not. R. Astron. Soc.* **153**, 453–469 (1971).
- ⁵ Smyth, M. J. & Wolstencroft, R. D. *Astrophys. Space Sci.* **8**, 471–477 (1970).
- ⁶ McGimsey, B. Q., Smith, A. G., Scott, R. L., Leacock, R. J., Edwards, P. L., Hackney, R. L. & Hackney, K. R. *Astron. J.* **80**, 895–922 (1976).
- ⁷ Selmes, R. A., Tritton, K. P. & Wordsworth, R. W. *Mon. Not. R. Astron. Soc.* **170**, 15–40 (1975).
- ⁸ Markova, L. T., Fomin, S. K. & Zhukov, G. V. *Astron. Circular* **791**, 1–2 (1973).
- ⁹ Markova, L. T. & Zhukov, G. V. *Astron. Circular* **843**, 1–2 (1974).
- ¹⁰ Deeming, T. J. *Astrophys. Space Sci.* **36**, 137–158 (1975).
- ¹¹ Barbieri, C., Romano, G., di Serego, A. S. & Zambon, M. *Astron. Astrophys.* (in the press).
- ¹² Kinman, T. D. *Science*, **162**, 1081–1085 (1968).
- ¹³ Angione, R. J. *Astron. J.* **78**, 353–368 (1973); thesis, Univ. Texas at Austin.

Small-amplitude, short-period waves in the E and F regions of the ionosphere

ACCURATE measurements of the virtual height of radio waves reflected from the ionosphere have been made by using a phase path ionosonde¹. This instrument determines the virtual height, h' , from the variation of echo phase with frequency, $\phi(f)$, since $h' = (c/4\pi)(\partial\phi/\partial f)$. Measurements are made in two ways. First, the ionosonde is swept in frequency and h' determined by taking the Fourier transform of the phase measurements on a number of frequencies. This is a form of pulse synthesis and very narrow pulses may be synthesised by combining measurements over a relatively wide frequency range¹. The second mode of operation is to repeatedly step the ionosonde through 20 frequencies in 0.4 s so that detailed time variations and height structure can be measured. Provided there are no overlapping echoes, virtual heights can be calculated by determining $\partial\phi/\partial f$ from adjacent frequencies. Suitable choices of frequency intervals can give very accurate values of h' (ref. 2). This paper presents results which show that small-amplitude (< 500 m peak to peak), short-period (~ 10 s) waves can occur in the E and F regions of the ionosphere.

Figure 1a shows an ionogram obtained by the phase path ionosonde using the pulse synthesis technique. A blanketing sporadic E layer is apparent and apart from effects due to interference at 2.5 MHz and 3.5 MHz, the virtual height is constant within 293 m over the frequency range 2–7 MHz. The value of 293 m corresponds to the quantisation level, of the pulse synthesis technique, used on this occasion. After recording this ionogram, the ionosonde was stepped from 2.0–3.9 MHz in 100-kHz steps. The variation of group path with time for several frequencies are shown in Fig. 2a. All frequencies show that in the 3 min for which data is presented, there is an overall

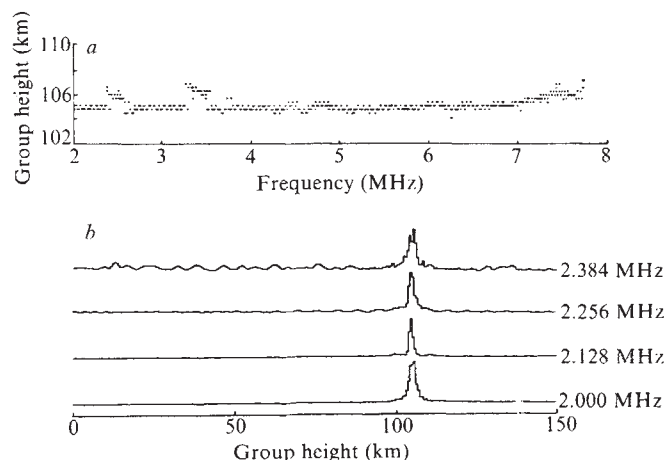


Fig. 1 *a*, An accurate ionogram produced by the phase path ionosonde using a pulse synthesis technique; *b*, examples of synthesised echoes.

rise and fall in the sporadic E layer. Superimposed on this, the three lowest frequencies show two bursts of oscillations. For the first, the period is about 11 s and the peak to peak amplitude is 240 m in virtual height. For the second burst, the period is 6.9 s and the peak-to-peak amplitude is 180 m. The oscillations are phase shifted in time on each of the frequencies and the period also varies slightly with the radio frequency. On 2.5 MHz the oscillations have decreased considerably in amplitude. Even though there is interference at 2.5 MHz, the group height can still be found since it is determined by calculating $\partial\phi/\partial f$ using frequencies on either side of 2.5 MHz. On 3.4 MHz the first burst of oscillation is not apparent and although oscillations occur at about the time of the second burst on the lower frequencies, the period and amplitude are different. Figure 2*b* shows similar data beginning 2 min later and lasting for 180 s. During this time the behaviour is in marked contrast to that shown in Fig. 2*a*. The sporadic E layer remained at an almost constant height and although there are small variations, there

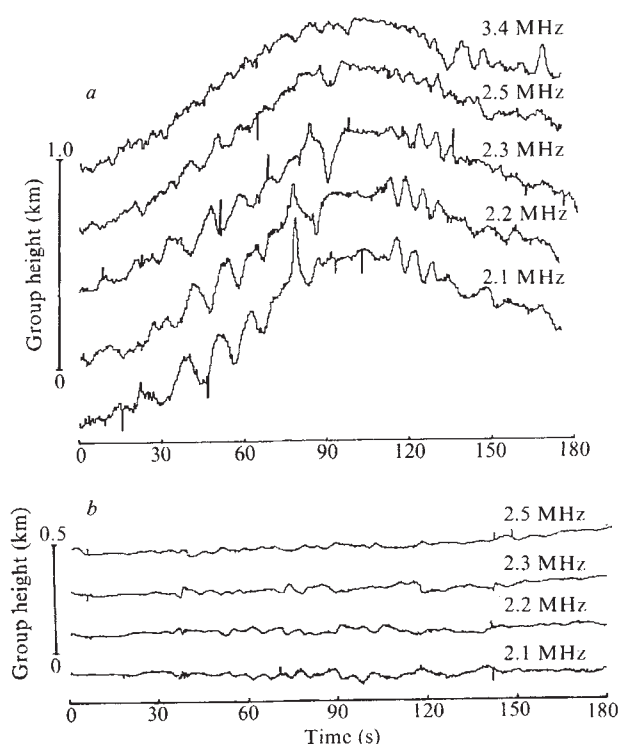


Fig. 2 *a*, The variation of group height with time on several frequencies reflected from sporadic E; *b*, similar data to *a* but beginning 2 min later.

are no pronounced oscillations of approximately constant period.

We are confident that the bursts of oscillation represent real effects associated with the sporadic E layer. Firstly, there is strong evidence that at an instant only one reflection point is involved. The reasons for this are that the sporadic E layer has such a constant virtual height over a wide frequency range and the synthesised echoes obtained for the ionogram in Fig. 1*a* do not show pulse spreading (see Fig. 1*b*). Secondly, if they were an artefact of the instrument (such as beating with the mains supply), the oscillations would appear on all frequencies and the time variations would be in phase and have the same period. The third point to be considered is that the antenna system used was not polarised so that there is no discrimination between ordinary and extraordinary propagation modes. The constant virtual height against frequency of the sporadic E layer indicates that there is relatively little ionisation below this layer, so that the virtual range of ordinary and extraordinary rays will differ only marginally. Since the extraordinary mode is strongly absorbed, our observations basically refer to the ordinary mode. However, one question to be answered is whether the variations shown in Fig. 2*a* could be caused by differential absorption effects. If such effects are due to time variations in the D layer, and, therefore, well below the reflection level, the time variations on the different frequencies should be in phase. If such effects occur near the reflection level then it means that the small-period oscillations are in fact occurring in the sporadic E layer.

The period of the oscillations is too short to be gravity waves but they could be sound waves. However, the fact that the oscillations occur only on a few frequencies suggests that they may be confined to a small part of the sporadic E layer and since they occur for only a short time, we may be observing bursts of irregularities produced by plasma instabilities occurring in the sporadic E layer. The amplitude of the oscillations is small but cannot be directly interpreted as the changes which are occurring in the real height of the reflection point because virtual height changes can be an overestimate of the true height changes and angle of arrival effects can occur as well.

Oscillations of short period (~ 4 s) and small amplitude (~ 250 m peak to peak) have also been observed in the F region but they are not so confined in height. Again they may well be the effects of either sound waves or plasma instabilities. Such short-period small-amplitude phenomena do not seem to have been detected in the ionosphere before and they are being studied further in order to explain them in detail.

This work has been supported by the Australian Research Grants Committee. One of us (J.C.D.) is the holder of a La Trobe University Research Scholarship.

J. C. DEVLIN
P. L. DYSON
P. R. HAMMER

Division of Theoretical and Space Physics,
La Trobe University, Bundoora,
Victoria, Australia

Received 13 April; accepted 1 June 1977.

¹ Devlin, J. C., Dyson, P. L. & Hammer, P. R. *Radio Sci.* (1977).

² Whitehead, J. D. & Malek, A. *J. atmos. terr. Phys.* 25, 599 (1963).

Radioactive ^{38}S in an atmospheric SO_2 and SO_2 oxidation rate measurement

BHANDARI *et al.*¹ and independently Perkins *et al.*² have detected cosmic-ray-produced ^{38}S in several precipitation samples. This paper reports on the first detection of cosmic-ray-produced radioactive ^{38}S as atmospheric $^{38}\text{SO}_2$. The use of this nuclide as a tracer for the *in situ* determination of SO_2 oxidation rates is also discussed. ^{38}S is produced by spallation of atmospheric argon and decays with a half life of 2.87 h by β^- -emission to ^{38}Cl ; 95% of the decays are accompanied by 1.88 MeV- γ -radiation, 5% undergo direct transition to the ^{38}Cl ground state.

The present work on the *in situ* measurement of SO₂ oxidation rates within the complex system of the natural atmosphere (where no other direct method to determine SO₂ conversion rates is known) began because we felt this could be a very interesting application of cosmic-ray-produced sulphur.

The radioactive sulphur, which after the spallation is present as sulphur dioxide, participates in the chemical fate of environmental SO₂ and is oxidised and converted to sulphate in the same manner and ³⁸SO₄²⁻ also decays. The ratio of radioactive SO₂ to radioactive SO₄²⁻ depends on the ratio of the radioactive decay rate to the SO₂ conversion rate. Thus simultaneous measurements of ³⁸S-radioactivity in SO₂ and in particulate, SO₄²⁻ should give information on the SO₂ conversion rates.

For sampling SO₂, atmospheric air is sucked, with a flow rate of about 150 m³ h⁻¹, through a paper filter (Macherey, Nagel & Co. MN 714) for removal of particulate matter and then through an absorption chamber. In this absorption chamber, a solution of 0.1 M NaOH and 1.5% H₂O₂ is sprayed into the air stream. More than 97% of SO₂ is absorbed by the dispersed solution. Within this solution SO₂ is oxidised and is precipitated as BaSO₄. Sulphate is reduced to H₂S by a procedure similar to that described by Polson and Strickland³, and by Stoeppler⁴. H₂S is directly used as counting gas in an internal-gas β-radiation detector. Details of the sampling procedure as well as of internal-gas counting will be published elsewhere.

In order to reduce detector background, and to discriminate against ³⁵S (half life 87 d; no γ-radiation) which is also present in atmospheric samples, low-level β-γ-coincidence technique as described by Roedel⁵ is used. The internal-gas counter tube of ~26 cm³ volume is fitted in the well of a 5 × 5-inch-NaJ(Tl)-scintillation crystal. The scintillation crystal registers the γ radiation and is gated by the β radiation detected in the gas counter. For the 1.88-MeV γ peak there is a detection efficiency of 7 ± 0.8% and a background counting rate less than 10⁻³ events min⁻¹.

Five samples have been measured between 5 February and 3 March 1976. The activities varied between 1 and 2 × 10⁻³ d.p.m. ³⁸S m⁻³, the mean being 1.3 × 10⁻³ d.p.m. m⁻³ (d.p.m. is disintegrations per minute). Sample volumes varied between 325 m³ and 520 m³. Due to the very poor counting statistics the uncertainties are still very great (about ±0.7 × 10⁻³ d.p.m. m⁻³).

Unfortunately, it is not yet possible to measure ³⁸S as airborne particulate ³⁸SO₄²⁻. As mentioned above a filter is used to retain and collect aerosols. This filter, however, causes also an appreciable retention of SO₂. This effect is most probably due to catalytic oxidation of SO₂ to SO₄²⁻, induced, for the most part, by the collected aerosols. The loss of SO₂ amounts to ~10% in new unloaded filters and to ~30–50% in filters loaded with aerosols. This indicates that ³⁸S-activity found on the filters is, for the greater part, due to a contamination by SO₂ converted within the filters. An improved sampling technique, however, is being developed which, together with an increased efficiency of radiation detectors, should allow simultaneous measurements of ³⁸SO₂ and ³⁸SO₄²⁻.

Finally, we shall give a rough estimation of the limits of the radiosulphur method. For this estimate only first-order reactions for the SO₂ conversion are allowed, further ³⁸S is assumed to be primarily present as SO₂ (this has still to be investigated), and transport and removal processes are ignored. Then the following equation for the ³⁸SO₂/³⁸SO₄²⁻ balance holds

$$d[{}^{38}\text{SO}_4^{2-}]/dt = +k[{}^{38}\text{SO}_2] - \lambda[{}^{38}\text{SO}_4^{2-}] \quad (1)$$

(λ and k are the constants of radioactive decay and of conversion, respectively).

In a steady state, equation (1) reduces to

$$k = \lambda[{}^{38}\text{SO}_4^{2-}]/[{}^{38}\text{SO}_2] \quad (2)$$

If a ratio ³⁸SO₄²⁻/³⁸SO₂ in the order of 0.1 can be detected, the minimum conversion rate which can be determined, is k = 0.1 λ (with λ = 0.242 h⁻¹) or about 2.5% h⁻¹. Laboratory experiments suggest that reaction rates for homogeneous SO₂ oxidation in urban air lie between 0 and 10% h⁻¹ (Cox and Penkett⁶, Cox⁷). Haury and Jordan^{8,9} report reaction rates for heterogeneous oxidation on the surface of aerosol particles up to 15% h⁻¹ depending on the relative humidity (Haury's and Jordan's measurements, however, are made at very high concentrations of SO₂ and aerosols).

The expression for k given above does not depend on the ³⁸S production rate. Nevertheless, the specific activity of ³⁸SO₂ may be affected by the vertical variance of the production rate. In the lower atmosphere, the spallation rate increases by a factor of ~2 with every 1.5 km up^{10,11}, a part of this elevated activity may be mixed downwards by eddy diffusion. The maximum additional increase of the ³⁸SO₂ concentration can be estimated by Fick's equation for the turbulent mixing

$$\frac{1}{[{}^{38}\text{SO}_2]} \frac{\partial [{}^{38}\text{SO}_2]}{\partial t} \bigg|_{\text{mix}} = \frac{1}{[{}^{38}\text{SO}_2]} \frac{\partial}{\partial z} \left(K_z \frac{\partial [{}^{38}\text{SO}_2]}{\partial z} \right) \\ \approx \frac{1}{[{}^{38}\text{SO}_2]_{z=0}} K_z \frac{[{}^{38}\text{SO}_2]_{z=z_0}}{z_0^2} \approx 2K_z/z_0^2 \approx 6.5\% \text{ h}^{-1} \quad (3)$$

(K_z being the coefficient of vertical eddy diffusion, which is assumed to be 2 × 10⁶ cm² s⁻¹, and z₀ = 1.5 km being the scale height for doubling the production rate, z is the vertical axis) or an increase of the ³⁸SO₂/³⁸SO₄²⁻ ratio of 20–25% within the lifetime of ³⁸S. This estimate, however, gives an upper limit for the error in the ³⁸SO₂/³⁸SO₄²⁻ ratio. In most cases one may assume an averaging within the scale of σ_z = √(2K_z/λ) ≈ 750 m rather than an appreciable increase of the ³⁸SO₂/³⁸SO₄²⁻ ratio.

Furthermore, the SO₂ to SO₄²⁻ balance in the real atmosphere may be affected by horizontal ventilation and by the uptake of SO₂ by plants and soils (see, for example, ref. 12 and references quoted therein).

W. ROEDEL
W. JUNKERMANN
L. LEIDNER

*Institut für Umweltphysik,
Universität Heidelberg,
D-6900 Heidelberg,
Federal Republic of Germany*

Received 12 November 1976; accepted 2 June 1977.

- Bhandari, N., Bhat, S. G., Kharkar, D. P., Krishna Swamy, S., Lal, D. & Thambane, A. S. *Tellus* **18**, 504 (1966).
- Perkins, R. W., Thomas, C. W., Hill, M. W. & Nielsen, J. *Nature* **205**, 4973 (1965).
- Polson, D. S. C. & Strickland, J. D. H. *Anal. Chim. Acta* **6**, 452 (1952).
- Stoeppler, M. *Nukleonik* **6**, 335 (1964).
- Roedel, W. *Nucl. Instr. Meth.* **61**, 41 (1968).
- Cox, R. A. & Penkett, S. A. *JCS, Faraday Trans. I*, **68**, 1735 (1972).
- Cox, R. A. *Tellus* **26**, 235 (1974).
- Haury, G. & Jordan, S. *Proc. Jahrestagung GAF, Bad Soden* (1973).
- Haury, G. *Rep. KFK 2318 UF Ges. f. Kernforschung, Karlsruhe* (1976).
- Lal, D. & Peters, B. *Handbuch Physik* **46/2** (Springer, Berlin, 1967).
- Roedel, W. *Naturwiss.* **59**, 456 (1972).
- Payrissat, M. & Beilcke, S. *Atmosph. Environ.* **9**, 211 (1975).

Stresses at spherulite boundaries

IN a recent study of drawing behaviour in polyethylene Barham and Keller¹ discussed the black lines which are often seen around spherulites of polyethylene viewed between crossed-polarisers. They state that these dark lines seem to be independent of their orientation with respect to the polarisation directions and attribute them to poorly crystalline regions of low molecular

weight material. Here I offer an explanation for these lines and show that they reveal the presence of stresses at the spherulite boundaries.

Figure 1 shows a 5- μm thick film of polyethylene ($\overline{M}_n = 11,100$, $\overline{M}_w = 68,000$) prepared by dropping a *p*-xylene solution on to a hot microscope slide, drying and melting the polymer and rapidly cooling. The dark lines surrounding the banded spherulites can be seen. Note that boundaries are not dark if they make an angle of between about 30° – 60° to the polarisation directions parallel to either edge of the figure. Rotation of the sample causes the dark bands to become as light as the rest of the spherulite, when the boundary is inclined at about 45° to the polarisation directions.

This suggests that the boundaries contain oriented polymer; but similar dark lines can be seen at boundaries between small, suitably oriented non-polymeric crystals. One explanation is that the dark band arises when light crosses the boundary during its passage through the specimen. The equations for polarised light passing through two superimposed crystal plates² show that extinction can occur when the slow and fast optical axes have a mirror symmetry across the boundary and the boundary is parallel to one of the polarisers. If the optical axes make an angle of 45° with the boundary the intensity I is given by $I = \sin^2[(\delta_1 - \delta_2)/2]$ where δ_1 and δ_2 are the two retardations. Thus, extinction will occur if the retardations are equal. If the angle of the optical axes to the boundary varies from 45° the system will still be close to extinction. If the boundary is tilted an arbitrarily small amount with respect to the light path, slightly different light paths will cross different thicknesses of the two crystal plates, and there will be one path where extinction occurs. The argument is more involved for banded spherulites such as polyethylene, but the result is still that an extinction line should be seen at the boundary and there is no reason to postulate the presence of a boundary film to explain the black line.

Further information can be derived by considering the regions of the spherulite where the boundary line zig-zags as can be seen in Fig. 1. Corresponding zig-zags can also be seen by using light Nomarski interference microscopy and scanning electron microscopy (SEM) though these lack the clarity and contrast of the polarised light views. Three origins for these zig-zags seem possible. The first is that the boundary may be tilted so much that the extinction position wanders from side to side. This idea is eliminated by observing these uneven boundaries by SEM and reflected light, and by the lack of any significant change as the sample is tilted during polarised light observation. The second possibility is that the two spherulites interpenetrate to produce a zone of changing birefringence. The observation of similar black lines between suitably oriented crystals of simple compounds shows that interpenetration is not necessary. The SEM and reflectance microscopy confirm the third possibility, that the zig-zags are real.

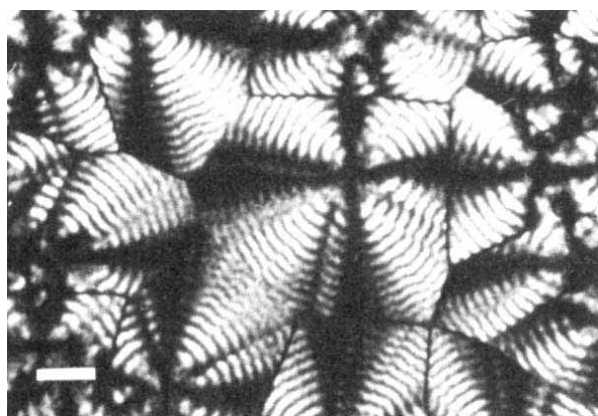


Fig. 1 Banded polyethylene spherulites showing zig-zag boundaries. Scale bar 10 μm .

These zig-zags occur where the band structures in the two spherulites intersect at an angle of about 90° and also where the banding is so out of phase that a dark band in one spherulite is crystallising adjacent to a light band in the other spherulite. In these circumstances the light band always protrudes into the dark one. Thus the light band, where the chains within the crystals are in the plane of the film and perpendicular to the spherulite radius³, grows faster than the dark band, where the chains are perpendicular to both the plane of the film and the spherulite radius. Since the crystal twisting frequency in angle per unit length is unchanged, the net result is that each spherulite alternately cuts across the front of the other then slows down as the lamellar crystals twist into the dark orientation. This is illustrated in Fig. 2. This effect is not restricted to thin films; the same zig-zag boundaries with the same light-dark relationship are observed in some regions of microtomed slices of bulk samples.

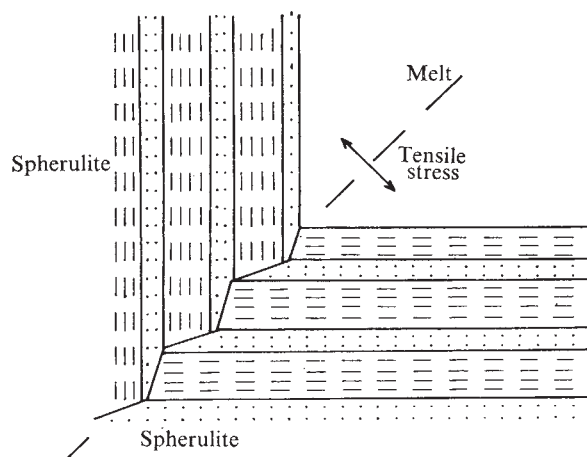


Fig. 2 Structure at a zig-zag boundary. Lined bands: bright, chains parallel to lines. Dotted bands: dark, chains perpendicular to paper.

In a bulk sample, when a microtomed section contains the centres of two spherulites and their common boundary, the plane of the boundary must be perpendicular to that of the section. The dark bands will then always contain crystals aligned with the chains parallel to the boundary and perpendicular to the section, while the chains in the bright bands are at an angle to the boundary.

The 10% shrinkage that occurs as spherulites form will produce a radial tensile stress and tangential compressive stress in the melt which will decay at a rate characterised by the melt relaxation time⁴. Where spherulites approach, the liquid layer trapped between the two may be unable to relax and a tensile stress perpendicular to the common boundary will develop.

The case where zig-zags form is shown in Fig. 2. Here the spherulite radii are both at 45° to the boundary and the banding is out of step. From studies on cross-linked polymers it is known that tensile stress increases the growth rate of crystals where the chain axis is parallel to the stress axis⁵. In Fig. 2 the tensile stress will have a component parallel to the chain axis in the light band but not in the dark band, so that the growth rate of the light band will be increased.

From the angle of the zig-zag we can make an order of magnitude estimate of the boundary stress. Taking the angle between the zig and zag to be about 60° we obtain a growth rate ratio of 1 : 1.5. Putting this into a thermodynamic theory of polymer crystal growth under stress⁶, taking the undercooling as 30°C and assuming that the stress does not affect the growth rate transverse to the stress we obtain a local strain of about 0.3 or a stress of about $6 \times 10^5 \text{ N m}^{-2}$ ($6 \times 10^6 \text{ dyn cm}^{-2}$). This can be taken as an average strain over a zone corresponding to the span of the zig-zag.

Black boundary lines having similar properties have been seen in several spherulitic polymers formed as thin films on

glass slides. In some cases non-birefringent dark lines were also seen. Barham and Keller have shown that the extent to which these lines can be seen depends on the molecular weight distribution and crystallisation conditions. Polyoxymethylene and poly(ethylene adipate) form banded spherulites where the boundaries are often irregular but do not show clear zig-zags. Residual boundary stresses of this magnitude must affect the strength and oxidation and corrosion resistance of polymers.

I thank A. Keller and P. J. Barham for their helpful comments.

P. D. CALVERT

The School of Molecular Sciences,
University of Sussex,
Falmer, Brighton, UK

Received 2 December 1976; accepted 16 May 1977.

¹ Barham, P. J. & Keller, A. J. *mater. Sci.* **11**, 27 (1976).

² Hartshorne, N. H. & Stuart, A. *Crystals and the Polarising Microscope* 4th edn, 302 (Edward Arnold, London, 1970).

³ Keith, H. D. & Padden, F. J., Jr *J. Polymer Sci.* **39**, 123 (1960).

⁴ Bolotov, I. E. & Novikova, L. P. *Kristallografiya* **21**, 163 (1976).

⁵ Mandelkern, L. *Crystallization of Polymers* (McGraw-Hill, New York, 1965).

⁶ Kobayashi, K. & Nagasawa, T. *J. Macromolecular Sci. (Phys)* **B4**, 331 (1970).

Transport of metal complex chlorides through plastic film membrane

THE acid-metal-chloro complexes of gallium and iron have been shown to be transported through polyurethane membranes by virtue of an acid or chloride gradient. Vofsi and co-workers¹⁻³ have reported on transport of uranium using complexing plasticisers in thin plastic films. More recently, the transport of sodium across a liquid membrane was described⁴. Recent work⁵ has demonstrated that open cell polyurethane foam sponge with surface areas of less than 0.1 m² g⁻¹ can dissolve gallium or iron as the HMeCl₄ complex to as much as 10% of the foam by weight. In this note, we investigate the transport of metal complexes, such as HMX₄ of gallium and iron, across polyurethane plastic films. We believe that the acid-chloro complex could dissolve in the film and so pass through such membranes.

Our all-glass diffusion apparatus consisted of two equal volume flanged cells with top openings for sampling. The 125-μm polyurethane film (ether type) was sealed vertically between the two glass flanges with high vacuum Silicone or Kel-F grease and clamped. Platinum electrodes sealed in glass were used to determine the specific conductance of the water (receiver) side which indicated the extent of migration of gallium from the loaded cell. The apparatus was immersed in a constant temperature bath controlled at 20±1 °C and the solutions were stirred with magnetic stirrer and Teflon-coated magnets. Small (1 or 2 ml) samples were periodically removed from the receiver side (Fig. 1) or both sides (Fig. 2) of the diffusion apparatus and analysed for acid and metal. Gallium was determined by AAS (ref. 6), whereas iron and uranium were analysed spectrophotometrically by the thiocyanate complex.

The effect of acid strength and chloride concentration on the extent of diffusion of gallium through the polyurethane membrane is shown in Fig. 1. The results for the diffusion of 200 p.p.m. gallium from 3.10 M HCl is not shown but after 500 h gave less than 1 p.p.m. gallium in the water cell which had a specific conductance of 0.17×10⁻³ Ω⁻¹ cm⁻¹. The rate of diffusion of gallium is qualitatively similar to the solvent extraction efficiency of the polyurethane foam^{5,7}, and is believed to be due to the equilibria



The depletion of gallium from the loaded cells was determined analytically and Ga was found to have decreased from

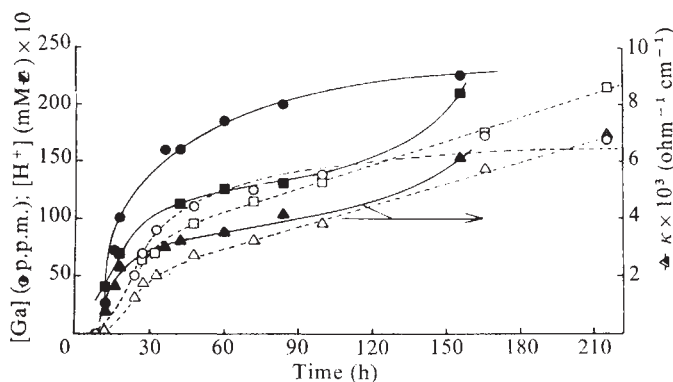


Fig. 1 Time dependence of specific conductance (κ , Δ) and diffusion of gallium ($\circ$, $\bullet$) and acid ($\square$, $\blacksquare$) through 9.1-cm² polyurethane membranes into 130 ml distilled water. Initial conditions: a, solid symbols, 200 p.p.m. Ga, 6.07 M HCl, 130 ml. b, Open symbols, 200 p.p.m. Ga, 3.12 M HCl, 3.00 M LiCl, 130 ml.

200 p.p.m. initially to less than 1 p.p.m. at the conclusion of the experiments. The final concentration of gallium in the receiver cell was, in some cases, greater than the initial 200 p.p.m. (the membrane initially separated equal volumes of solution) because of an osmotic effect which was noticed when most of the gallium had diffused through the membrane. The osmotic effect depleted water from the water cell causing the volume of the loaded cell to increase. The volume of water transported was about 10–20% of the total volume. This was primarily accommodated by the bulging of the membrane into the water cell.

The mechanism of transport is readily explained by equations (1) and (2). The HGaCl₄ complex is formed in the acid (loaded) cell dissolves in the membrane through which it can diffuse. As the complex appears on the water (receiver) side it dissociates back to the component ions, H⁺, Ga³⁺ and Cl⁻. The Ga³⁺ ion cannot diffuse back until the concentration of the HCl has increased significantly to reform the HGaCl₄ complex. This back diffusion has been observed with iron (III) where it was also noted that the initial hydrolysis of the HFeCl₄ complex in

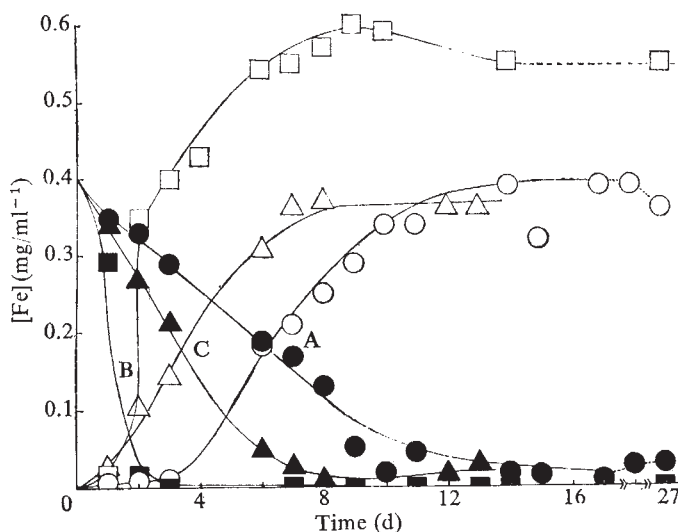


Fig. 2 Diffusion of iron from loaded cell (solid symbols) through polyurethane membranes into receiver cell (open symbols) as a function of time. a, Circles: 620 ml of 0.4 mg ml⁻¹ Fe³⁺, 2 M HCl, 5 M LiCl with 620 ml of 2 M HCl in receiver cell, membrane area 42 cm². b, Squares: 300 ml of 0.4 mg ml⁻¹ Fe³⁺, 2 M HCl, 5 M LiCl with 300 ml of 0.1 M HCl in receiver cell, membrane area 21 cm². c, Triangles: 750 ml of 0.4 mg ml⁻¹ Fe³⁺, 2 M HCl, 5 M LiCl, with 750 ml of 2 M HCl in receiver cell, membrane area 50 cm². Cell preconditioned with HCl and LiCl for 6 d before Fe³⁺ was added.

water precipitated the red-brown $\text{Fe}(\text{OH})_3$ which was deposited on the water side of the membrane. The precipitate then dissolved slowly as the acid diffused through the membrane. This may have occurred for gallium but was not noticed because the $\text{Ga}(\text{OH})_3$ is not coloured. The formation of the $\text{Fe}(\text{OH})_3$ indicates that the acid-metal complex diffuses through the membrane faster than HCl.

When a low-acid (3 M HCl) gallium system was studied with added LiCl (3 M) the diffusion rate was much faster than for 3 M HCl but not as fast as for 6 M HCl. This is consistent with the formation of the complex and its solubility in polyurethane⁹. On completion of the experiment (230 h) (Fig. 1 b), analysis for lithium by AAS in the water cell showed that less than 1 p.p.m. may have diffused through the membrane.

The results for the diffusion of iron (III) through the membrane are shown in Fig. 2, where the receiver cell contained 2 M and 0.1 M HCl to prevent the hydrolysis of the iron complex to insoluble hydroxide. The rate of diffusion of the iron is greatly affected by the strength of the acid in the receiver cell. The time lag noted for the appearance of metal in the receiver cell was significantly reduced when the membrane was conditioned for six days in the cell with acid and LiCl before the iron was added (Fig. 2 c). This implies that the mechanism of transport of the metal is primarily via the anion complex, MCl_4^- , which jumps from one protonated ether group to another. The membrane morphology had changed considerably during the diffusion experiments and the appearance of blisters, vacuoles, resemble the changes observed by Vofsi⁸ with plasticised membranes.

The diffusion of iron (III) chloride in 6 M HCl solution was tested with membranes of 50 μm polyethylene, 25 μm Saran, 25 μm PVC and 50 μm Teflon. In all these cases no detectable iron had diffused through the membrane in 15 days. Iron (II) chloride in 6 M HCl did not diffuse through the polyurethane membrane whereas iron (III) bromide in HBr solution was slightly slower than the iron (III) chloride. Preliminary experiments with $\text{UO}_2(\text{NO}_3)_2$ in the presence of $\text{Al}(\text{NO}_3)_3$ showed diffusion of uranium to be occurring through polyurethane membrane.

We are grateful to the Manitoba Research Council and the National Research Council of Canada for financial support.

H. D. GESSER
G. A. HORSFALL
K. M. GOUGH
B. KRAWCHUK

Department of Chemistry,
University of Manitoba,
Winnipeg, Canada

Received 24 March; accepted 25 May 1977.

- Block, R., Finkelstein, A., Kedem, O. & Vofsi, D., *Ind. Eng. Chem. Process Des. Develop.* 6, 231 (1967).
- Block, R., Katchalsky, A., Kedem, O. & Vofsi, D., *U.S. Patents No.* 3,450,630; 3,450,631; June 17 (1969).
- Vofsi, D. & Jagur-Grodzinski, J., *Naturwissenschaften* 61, 25 (1974).
- Choy, E. M., Evans, D. F. & Cussler, E. L., *J. Am. chem. Soc.* 96, 7085 (1974).
- Gesser, H. D., Bock, E., Baldwin, W. G., Chow, A., McBride, D. W. & Lipinsky, W., *Separation Sci.* 11, 317 (1976).
- Chow, A. & Lipinsky, W., *Anal. Chim. Acta* 75, 87, (1975).
- Horsfall, G. A. thesis, University of Manitoba, (1977).
- Marian, S., Jagur-Grodzinski, J., Kedem, O. & Vofsi, D., *Biophys. J.* 10, 901 (1970).

Long-period effects in the Romanian earthquake of March 1977

THE field study of the Romanian earthquake of 1977 suggests that strong ground motions, for engineering purposes, may differ considerably from those currently adopted for design on the basis of US West Coast-type of recordings. The earthquake of March 4 1977 in Romania had a magnitude of $M = 7.2$ and a depth of about 90 km. It originated from the source of

intermediate-depth earthquakes of Vrancea, beneath the bend of the Carpathian mountain arc, at 45.8°N , 26.7°E , a source similar to that of intermediate events in the Hindu Kush.

It was felt over an area of $1.3 \times 10^6 \text{ km}^2$ and caused damage over an area of about $80,000 \text{ km}^2$ within which the most frequently occurring intensity did not exceed VII (MM). It mostly affected the densely populated and rapidly developing centres of Craiova, Pitesti, Zimnicea, Bucharest, Ploesti, Focsani, Birlan and Iasi, where it caused damage worth over 10^9 \$ US. The earthquake killed about 2,100 people of whom 1,800 perished in Bucharest (BU), and injured 10,500, 2,300 seriously. Much of the damage was caused to old multi-storey reinforced concrete buildings of 6–12 storeys. Some of them had been damaged in 1940 by an earthquake of almost identical characteristics and by the war. Relatively few modern high-rise constructions suffered serious structural damage.

In Bucharest, at a focal distance of 190 km, the shock triggered an SMAC-B strong-motion accelerometer and a Wilmot seismoscope in the basement of a single-storey structure founded on thick, compact alluvium. The SMAC record shows that the strongest shaking began about 19 s after the instrument started to record, and this strongest shaking consisted of a double acceleration pulse in the two horizontal components of 154 and 206 gal respectively that contain more than 80% of the total energy flux of the event. Figure 1 shows the acceleration response spectrum (S_a), for 5% critical damping, of the main portion of the north-south component of motion¹. It also

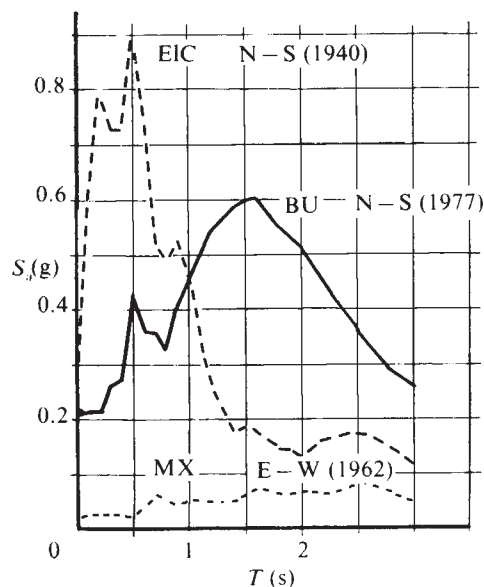


Fig. 1 Acceleration response spectrum of the main portion of the north-south component of motion in the Bucharest earthquake. The ground motions produced by other earthquakes in El Centro and Mexico City are shown for comparison.

shows the response spectra of two other ground motions produced by earthquakes of roughly the same magnitude, in the near-field (El Centro, EC) and in the far-field (Mexico City, MX) for comparison. The former occurred on 19 May 1940 at a depth of about 7 km, had a magnitude $M = 7.1$ and was recorded on dense alluvium at a focal distance of 14 km. The latter occurred on the 11 May 1962 at a depth of about 40 km, had a magnitude $M = 7.0$ and was recorded on very soft and thick deposits of high water content at Mexico City, at a focal distance of 270 km. The difference in shape of the response spectra between far-field (BU and MX) and near-field (EC) as well as the shift of the spectral maxima is obvious. This difference is more apparent in Fig. 2 where the response spectra of Fig. 1 have been normalised with respect to their corresponding zero period accelerations. Allowing for the larger magnification in the MX record for periods longer than 2 s, due to layer

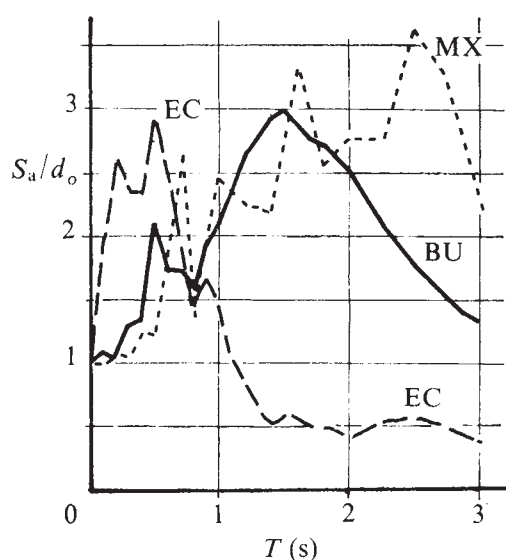


Fig. 2 Normalised spectra from Fig. 1 with respect to their corresponding zero period accelerations.

response, all three events show the same maximum amplification of about three times their zero period acceleration but at distinctly different periods, much longer for the far-field motions.

There is, of course, insufficient evidence to claim that the (BU) spectra are representative of an intermediate depth, medium magnitude earthquake. They simply imply that in this particular case the focal mechanism and preferential attenuation at higher frequencies along the path produced intense ground motions at unusually high periods.

These long-period characteristics of intermediate-depth earthquakes from the Vrancea region are reflected also on the seismoscope recordings. In Bucharest, the recording of the Wilmot seismoscope shows a double amplitude of 41 mm which is consistent with 44-mm amplitude response of the SMAC-B record at $T = 0.75$ s and 10% damping. At Galati, at a focal distance of 140 km the double amplitude was 27 mm; at Nis and Skopje in Yugoslavia, at distances of 480 and 610 km, the Wilmot amplitudes were about 6 and < 1 mm respectively. Other earthquakes originating from Vrancea, those of 2, 15 October and 14 December 1966 of magnitude 5.6, 5.4 and 5.2 produced seismoscope amplitudes at Ksihinev in the USSR, at a focal distance of 260 km, of 1.7, 1.4 and 0.7 mm respectively.

The greater effect of the 1977 earthquake in Bucharest was on flexible, 6–12-storey high reinforced concrete frame structures of low ductility without shear walls. These structures have a fundamental period of the order of 0.7–1.6 s, which places them on the ascending branch of the BU spectrum. Progressive damage during the earthquake should have caused a lengthening of their period and an increase in the lateral forces acting on them, whereas for the EC spectrum just the opposite is true. In contrast, rigid structures of large panel or frame construction with shear walls, of the same height, as well as 1–3-storey masonry dwellings, suffered little damage. These structures have much shorter periods of the order of 0.2–0.7 s, and they belong to the early, more subdued part of the BU spectrum. Had the ground motions in Bucharest been of the EC type, the greater effects of the earthquake should have been on the more rigid structures.

It is evident, therefore, that the adequacy of current lateral force code requirements derived from near-field ground motions, may be insufficient for flexible, low ductility structures wherever there exists the potential of a large, distant or intermediate-depth earthquake. This situation may arise more often than previously believed, particularly in the eastern Mediterranean region, and in fast developing cities at some distance from

major throughgoing faults capable of producing large earthquakes.

N. N. AMBRASEYS

Department of Civil Engineering,
Imperial College of Science and Technology,
London SW7, UK

Received 22 April; accepted 13 June 1977.

¹ Brune, J. N. *The Physics of Earthquake Strong Motion* (eds Lomnitz, C. & Rosenblueth, E. (Elsevier, Amsterdam 1976).

Confirmation of central volcanoes off the Icelandic coast

THE Icelandic shelf and slope region occupies much of the transition zone between the complex Icelandic hot spot and more uniform ocean-ridge areas to the north and south. This transition zone is relatively little known geologically, but in this communication we summarise some recent observations on the nature of its basement. It is well known¹ that the shelf is generally narrower off the eastern half of Iceland than off its western half. This is most obvious south of Iceland, where the shelf width changes suddenly at the eastern volcanic zone; the insular slope is also steepest off south-eastern and north-eastern Iceland. Recent geophysical surveys^{1,2} in the southern and south-eastern shelf area have indicated the presence of an abrupt basement scarp now covered by sediments, 5–14 km inside the bathymetric shelf break (Fig. 1). This scarp is at least 1 km high and only a few km in mean width. No such basement scarp is indicated in comparable surveys conducted so far off western Iceland^{1,3}; north-east of the island, detailed data is not yet available.

A causal relationship between this and other observed east–west asymmetries in the geology of Iceland, including the much greater uplift and erosion in eastern than in western Iceland since the Upper Tertiary, is likely to be found in differences in crustal thickness or upper mantle composition respectively east and west of the eastern volcanic zone. Similarly, the abrupt change in the chemistry of recent volcanics occurring north of Iceland⁴ very close to the shelf break, may directly reflect changes in crustal thickness⁵, rather than damming effects of the Tjörnes fracture zone on subcrustal flow⁴.

Another point concerns the central volcanoes of Iceland, first described by Walker⁶, which are of major significance in its geological evolution. Over 60 such centres, active or extinct, have now been identified on the island. From the results of geophysical surveys^{3,7} it has been concluded that several central volcanoes occur on the insular shelf off eastern and western Iceland, and that the trace of the Icelandic hot spot in the North Atlantic may coincide with the greatest density of such centres.

In an attempt to confirm the occurrence of central volcanoes on the Icelandic shelf, we have dredged at three sites (Fig. 1) where topographic highs are associated with the major magnetic anomalies believed to be characteristic for such volcanoes. The dredging was carried out by the RV *Hafthor*, of the Marine Research Institute, in November 1975, and a collection of igneous rock boulders was recovered at each dredge site.

Those features of a dredge collection which might be used to indicate the presence of a central volcano include (1) a large proportion of acidic, intermediate or gabbroic rocks (2) rocks having suffered intense hydrothermal alteration, and (3) unusually magnetic and magnetite-rich rocks. Accordingly, the rock types in these dredge samples were identified in thin section, and their magnetic susceptibility and natural remanence were measured in core specimens. The results (to be published in more detail⁸) may be summarised as follows.

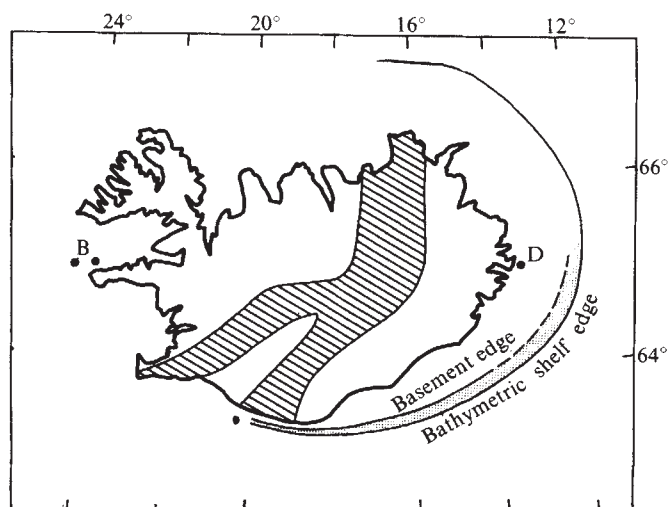


Fig. 1 Map of Iceland, showing the smoothed position of the upper edge of the steep insular slope off the eastern half of the island, and a major basement edge inferred from marine magnetic and gravity data off southern and south-eastern Iceland^{1,2,7} to occur under shelf sediments. Both edges are indistinct between 64° and 65°N. Hatched areas: active volcanic zones in Iceland. B, D, Dredge localities.

Of the two dredge sites off western Iceland (20 km apart), one yielded six basalt boulders and six rhyolite boulders, while the other yielded 71 basalt and diabase boulders and two rhyolites. Alteration state and magnetic properties of these samples are similar to those found in lavas on shore.

At the Diönubodi dredge site 14 km off Gerpir promontory, eastern Iceland, the samples recovered were predominantly (21 out of 35) of acid and intermediate rock types, including granites, rhyolites, acid tuff, and andesites. Two samples contain epidote. The magnetic properties of this collection are also unusual: thus, nine specimens out of the 34 measured have volume susceptibilities exceeding $0.1 \text{ T A}^{-1} \text{ m}$ ($8 \times 10^{-3} \text{ G Oe}^{-1}$), compared with only 1% of the specimens in a collection from 740 Eastern Iceland basalt lavas recently measured by us.

We are confident that the Diönubodi dredge collection can only be derived from a local central volcanic complex, which also causes the large (up to 6,000 nT) magnetic anomaly⁷ observed there. It is, furthermore, difficult to explain the high proportion of acid rocks at one of the western sites without recourse to similar conditions. These findings strongly support previous hypotheses^{3,7} on the occurrence of other central volcanoes on the Icelandic shelf.

L. KRISTJANSSON

Science Institute, University of Iceland,
Dunhaga 3, Reykjavik, Iceland

K. THORS

Marine Research Institute,
Skúlagata 4, Reykjavik, Iceland

H. R. KARLSSON

Department of Engineering and Natural Sciences,
University of Iceland, Dunhaga 3,
Reykjavik, Iceland

Received 5 April; accepted 1 June 1977.

¹ Palmason, G. in *Geology of Continental Margins* (eds Burk, C. & Drake, C.) 375–379 (Springer-Verlag, Berlin, 1974).

² Kristjánsson, L. *Marine geophys. Res.* 2, 315–326 (1976).

³ Kristjánsson, L. *Marine geophys. Res.* 2, 285–289 (1976).

⁴ Brooks, C. K. & Jakobsson, S. in *Geodynamics of Iceland and the North Atlantic Area* (ed. Kristjánsson, L.) 139–154 (Reidel, Dordrecht, 1974).

⁵ Sigvaldason, G. E., Steinthorsson, S., Oskarsson, N. & Imslund, P. *Bull. Geol. Soc. France* 18, 863–867 (1976).

⁶ Walker, G. P. L. *Q. J. geol. Soc. Lond.* 119, 29–63 (1963).

⁷ Fleischer, U., Holzkamm, F., Vollbrecht, K. & Voppel, D. *Deut. Hydrogr. Z.* 27, 97–113 (1974).

⁸ Kristjánsson, L., Thors, K. & Karlsson, H. R. *Natturfraedisingurinn* 46 (in the press) (Icelandic, with English abstract).

How do children learn the meanings of verbs?

ONCE children have begun to learn their native tongue, they acquire vocabulary at a remarkable rate. An intelligent 6-yr-old is able to recognise 13,000 words, and more than double that number 2 yr later¹. According to orthodox linguistic theory, the learning of verbs and other relational terms ought to be difficult because, in addition to their meaning, they have 'selectional restrictions', which constrain the noun phrases that can occur with them². A child must learn, for example, both the meaning of the verb 'fly' and the fact that it takes subjects which are restricted to denoting moveable objects. This linguistic theory has, however, been criticised, and it may be a misleading basis for a model of language acquisition since meaning and selectional restrictions can hardly be independent³. In particular, the subjects and objects that occur with a verb might help the child to infer its meaning, and likewise the meaning of a verb might help to define its possible subjects and objects.

In order to investigate such assumptions, we provided 15 young children (mean age, 3.10 yr) with an opportunity to learn some verbs. On each trial, the experimenter told a story to one of the children and acted it out with puppets. A typical story ran as follows: 'John stepped out of the boat and the water mibbed his trousers so he went home to change into some dry clothes. The water had mibbed his trousers right through so Simon made him some hot tea. But John dropped his cup and the tea mibbed over the floor'. The novel verb, mib, has a transitive meaning similar to that of soak and an intransitive meaning similar to that of spill—there is no single word corresponding to it in English, but it always occurred with a subject noun phrase denoting a liquid. The child heard the story four times: on the first two occasions the experimenter acted it out appropriately, and on the second two occasions the child attempted to do so. As a test of what the child had learned, he or she was presented with four items unrelated to the story (for example a female doll, some orange juice in a container, a car, a ball) and asked 'Which one can mib?' Only one item—the orange juice—is in accord with the selectional restrictions of the verb. Any child who made a correct choice was retested 7–10 d later. In this retrial, only the test was carried out: the child was simply asked to pick out the item that mibbed from a new set of four.

Four different stories were devised in order to test the children's ability to acquire four sorts of verb defined in terms of their selectional restrictions: animate subject, animate object, liquid subject, liquid object. Earlier studies of children's comprehension of sentences suggested that animate subject and liquid object verbs should be easier to learn than liquid subject and animate object verbs^{4,5}. A fifth story was also used for reasons that will become apparent shortly. None of the novel verbs had any single semantic counterpart in English. They were based on five nonsense syllables, with association values of approximately 2.1 (ref 6), which were allocated to the stories in a counterbalanced way from one subject to another. Each child was individually tested with each of the stories in five separate sessions during the course of a morning. The order of presentation of the stories was randomised.

The children picked out a mean of 2.4 correct items on the five initial tests (with eight children performing significantly better than chance). There were 33% correct responses for animate subject verbs, 66% for animate object verbs, 60% for liquid subject verbs, and 46% for liquid object verbs. These differences fail to support the view that animate subject and inanimate object verbs should be easier to learn, and were not significant (Cochran's $Q = 2.42$, $P > 0.50$). A week later, the children who had responded correctly to a verb were retested with it, and the percentages of correct responses were: 76% for animate subject verbs, 66% for animate object verbs, 12% for liquid subject verbs, 28.5% for liquid object verbs. The animate

verbs were significantly better retained than the liquid verbs (Fisher-Yates exact probability, $P = 0.01$, on the assumption that all the data were independent).

Selectional restrictions probably involve an inference about whether a particular referent is a possible subject of a verb, and so on'. Hence, in the case of a sentence such as, 'The Smiths saw the Rocky Mountains flying to California', a child who lacks some pertinent general knowledge about mountains or relative motion will be unable to make the required inference and may well conclude that the Rocky Mountains were flying. In order to test this hypothesis, a fifth story was constructed with a nonsense verb that selects subjects capable of movement. As a test of what had been acquired, the children were asked to act out two sentences: 'Jane saw the boat gebbing towards the ball', and 'John saw the house gebbing towards the park'. Their initial performance was 93% and 33% correct, respectively. When we retested the successful children a week later, performance was 100% and 50% correct, respectively. Evidently, it was easy for the children to learn that the subject of the nonsense verb denoted something that moved, but few children made the intended interpretation of the second test sentence. Its syntax suggests that the house is the entity that gebs and, in fact, 10 subjects attempted to move the house. They did not infer that the house cannot move with respect to the park. One subject, however, put the doll in front of the house with his hands in the air and explained: 'He's stopping the house from gebbing to the park'.

At least half the children learned something about the selectional restrictions on verbs from the stories they heard, which suggests that this experimental procedure is useful. But it does provide two clues to the meanings of the verbs: the accompanying actions and the linguistic context. A second study was accordingly carried out in which 14 children (mean age 4.3 yr) merely listened to the stories (4 times), which were not accompanied by any actions. Performance on the initial tests corroborated the results of the previous study: 11 subjects made two or more correct responses which, overall, were very evenly distributed across the four sorts of verb. There was also no significant difference, however, between the verbs on the retest a week later.

Young children are evidently capable of picking up incidental information about a variety of different sorts of verb. In previous studies of comprehension, selectional information has often been uncontrolled. For example, it has been found that 5-yr-olds tend to answer the question, 'Is this doll easy to see?', as though the doll were the subject of the verb. It remains unclear whether the response reflects a genuine syntactic bias⁸ or merely the fact that the major selectional restriction of the verb clearly points to an entity with eyes.

Our results suggest that children are sensitive to whatever cues are available to meaning. They readily pick up selectional information about both animacy and liquidity. With hindsight, it might be odd if this tacit skill were exercised to a significantly different extent with such familiar concepts. Their ability to retain newly acquired knowledge may, however, depend on its nature and on the sort of experience leading to its acquisition. The answer to our question does, indeed, seem to be that children learn the meanings of verbs rapidly, without their attention being deliberately drawn to them, and often in circumstances where the major cue is the selectional information provided by the sentence as a whole.

We thank David McNeill for his advice and also his son, Randy, who was the first subject to grapple with the Rocky Mountains flying to California.

TIL WYKES
P. N. JOHNSON-LAIRD

Laboratory of Experimental Psychology,
University of Sussex,
Brighton, UK

Received 14 April; accepted 8 June 1977.

- ¹ Templin, M. C. *Certain Language Skills in Children: Their Development and Interrelationships* (University of Minnesota Press, Minneapolis, 1957).
- ² Katz, J. J. & Fodor, J. A. *Language* 39, 170-210 (1963).
- ³ Savin, H. B. *Cognition* 2, 213-238 (1973).
- ⁴ Jarvella, R. & Sinnott, J. J. *verb. Learn. verb. Beh.* 11, 47-53 (1972).
- ⁵ Howe, H. E. & Hillman, D. J. *verb. Learn. verb. Beh.* 12, 132-139 (1973).
- ⁶ Noble, C. E. *Psychol. Rep.* 8, 487-521 (1961).
- ⁷ Miller, G. A. & Johnson-Laird, P. N. *Language and Perception* (Cambridge University Press, London, 1976).
- ⁸ Chomsky, C. *The Acquisition of Syntax in Children from 5 to 10*. (MIT, Cambridge, Massachusetts, 1969).

Adoption study supporting genetic transmission in manic-depressive illness

COMPARISON of adoptive parents of persons with a psychiatric disorder with their biological parents provides a unique opportunity to separate the interacting aetiological roles of heredity and environment. We have conducted such a study in manic-depressive illness; the results strongly support the importance of genetic factors in the transmission of this disorder.

A genetic vulnerability to manic-depressive disorder has been demonstrated by family, twin, and linkage studies¹⁻⁴. To our knowledge, this is the first report of an adoption study of affective illness, but research involving adoptive families has been done for schizophrenia and other psychiatric disorders⁵⁻¹⁰. Our investigation was carried out in Belgium because adoptive registers are available there, and the adoption agencies were willing to help us to obtain subjects.

Our subjects were the adoptive and biological parents of manic-depressive adoptees. There were three sets of controls: (1) the parents of manic-depressives who were not adoptees; (2) the adoptive and biological parents of normal adoptees, and (3) the parents of individuals who had contracted poliomyelitis during childhood or adolescence. This last group was designed to control for the effect on parents of bringing up a disabled child.

The group of affected offspring (that is, manic-depressive adoptees) was ascertained by systematically examining the medical records of five outpatient clinics and five inpatient services in the vicinity of Brussels. All admissions during a 5-yr period (1971-1976) were reviewed. Those persons who were designated as manic-depressive and who were described as having been adopted were interviewed by one of us (J.M.) to

Table 1 Demographic characteristics of the offspring

	Index cases Manic- depressive adoptees N = 29	Manic- depressive non-adoptees N = 31	Normal adoptees N = 22	Polio N = 20
Ascertainment				
Adoption Agency	—	—	12	—
Inpatient	19	17	—	—
Outpatient	10	14	—	20
Age (mean, yr)	32.41	32.35	32.00	29.25
Sex				
Male	10	9	6	5
Female	19	22	16	15
Age at adoption (mean, in months)	5.41	—	6.00	—
Age of onset (mean)	22.07	24.26	—	8.35
National origin				
Flemish	16	18	13	13
Walloon (French)	13	13	9	7
Religion				
Catholic	25	26	22	17
Protestant	4	5	0	3
Socio-economic status*				
Education (mean)	3.83	3.90	3.86	3.80
Occupation (mean)	3.90	4.00	3.95	3.80
Marital Status				
Married	16	21	16	8
Single	10	5	4	12
Divorced	3	5	1	0
Separated	0	0	1	0

* Hollingshead-Redlich scale¹³.

Table 2 Demographic characteristics of the parents

	Adoptive of manic-depressive adoptees (N = 29)		Biological of manic-depressive adoptees (N = 31)		Adoptive of normal adoptees (N = 22)		Biological of polio patients (N = 20)	
	♂	♀	♂	♀	♂	♀	♂	♀
Number living	29	28	29	28	21	21	19	20
Age (mean, yr)	61.9	61.2	56.8	55.7	59.1	58.1	52.8	50.3
Socio-economic status†								
Education			3.86					
Occupation			3.93					
Marital Status								
Married to each other			29					
Married to different spouses			0	(15)				
Never married			0	2		14		
Divorced			0			5		
Separated			0			0		

* Figures in parentheses are totals.

† Hollingshead-Redlich Scale¹³.

confirm the diagnosis. We used a modified form of the semi-structured interview, Current and Past Psychopathology Scales (CAPPS) (ref. 11) and the diagnostic criteria of Feighner *et al.*¹². Only persons who had bipolar illness (that is, had experienced both manic and depressive episodes) were accepted as probands. To meet our criteria, they also had to have been transferred to their adoptive homes before 1 yr of age and to have been raised by those parents until adulthood. The adoption agencies supplied the details on the dates of adoption of the offspring, and gave us the names of the biological parents. Municipal registers were used to locate all parents. We only accepted families in which at least three of the four parents were available and willing to participate.

Manic-depressive offspring who were not adopted were ascertained from admissions records of the same facilities as the adoptees, covering a similar time period. Psychiatrically normal adoptees were ascertained through the adoption agencies; these individuals were given clinical interviews and only those without psychopathology were accepted. Their biological and adoptive parents were traced by the same procedure as those of the experimental group. None of the adoptive parents were related to the biological parents. The poliomyelitis patients were ascertained through three paediatricians who had been treating them over a long period. The three groups of control offspring were selected out of the total number of possible subjects by matching them with the index population according to age, sex, national origin, religion, and socio-economic status. Table 1 summarises the ascertainment and demographic characteristics of the experimental and control groups.

All of the parents were interviewed by one of the clinicians, blind as to the adoptive and clinical status of the offspring. The same instruments and criteria were used as for the diagnostic confirmation of their offspring. The interviews took place in the parents' homes unless the parents preferred to meet at the local

centre. The greatest distance travelled was 52 miles from Brussels, indicating a low mobility of this sample. Two and a half to three hours were spent with the parents for the psychiatric interview. The parents were instructed at the outset not to speak about their children until specifically asked. Demographic characteristics of the parents are summarised in Table 2.

The major finding of this investigation is that psychopathology in the biological parents is in excess of that found in the adoptive parents of the same manic-depressive offspring (Table 3). If we focus on disorders which we may call the affective spectrum, namely, bipolar affective disease (episodes of mania and depression), unipolar affective disease (psychotic depressions without mania), schizoaffective psychosis (schizophrenic and affective episodes), and cyclothymia (cyclothymic personality), the difference is significant at the level of $P < 0.025$ ($\chi^2 = 5.10$). Previous genetic studies by us and others support the inclusion of unipolar and schizoaffective disorders as genetically related to bipolar illness when they are found in close relatives of bipolar patients^{2,4,14-15}. If we include other forms of psychopathology, the difference is highly significant ($\chi^2 = 7.29$, $P < 0.01$). The frequency of non-affective psychopathology was no higher in the biological parents of bipolar adoptees (9%) than in those of normal adoptees (16%), indicating that it is by virtue of the affective disorders that the former differ from the latter (31% compared with 2%).

Table 3 also shows that the degree of psychopathology in the biological parents of manic-depressive adoptees is similar to that of the parents of the non-adopted manic-depressives, and that the rate of psychiatric disorder in the adoptive parents of the experimental group is similar to that of the adoptive parents of the normal offspring group. The degree of total psychopathology in the biological parents (of normal offspring) who gave their children for adoption is slightly greater than in adoptive parents who brought up those same individuals. It is

Table 3 Diagnosis of the parents

	Bipolar adoptees (N = 29)		Bipolar nonadoptees (N = 31)		Normal adoptees (N = 22)		Poliomyelitis (N = 20)	
	Adoptive parents	Biological parents	Adoptive parents	Biological parents	Adoptive parents	Biological parents	Adoptive parents	Biological parents
	♂	♀	♂	♀	♂	♀	♂	♀
Bipolar	1	0	3	1	0	0	0	0
Unipolar	3	3	1	11	1	2	3	1
Schizoaffective	0	0	0	2	0	0	0	0
Cyclothymic	0	0	0	0	1	0	0	0
Affective spectrum	4	3	4	14	2	2	3	1
Percentage	14	10	14	48	10	10	15	5
Schizophrenia	0	0	0	0	0	1	0	0
Alcoholism	2	0	2	1	0	0	1	0
Sociopathy	0	0	1	1	0	0	0	0
Other	0	0	0	0	0	0	0	0
All psychopathology	6	3	7	16	2	3	4	1
Percentage	21	10	24	55	9	14	20	5

* Figures in parentheses are totals.

† $\chi^2 = 5.10$, $P < 0.025$.‡ $\chi^2 = 7.29$, $P < 0.01$.

worth noting that this difference is due to an excess of alcoholism and sociopathy in the former group. Finally, the degree of psychopathology in the parents of polio patients is in the same range as in both groups of adoptive parents. All these findings support our conclusion that our experimental sets of parents are truly representative of the degree of psychiatric disorder which is present respectively in those parents who bring up and those who contribute genetically to manic-depressive individuals.

Another finding of interest is that no father-to-son transmission of manic-depressive illness was seen in our entire sample. This is consistent with a sex-linked model of bipolar illness, which has been suggested by some previous genetic studies²⁻⁴. Finally, bipolar illness, as would be expected, had an early onset in all parents in whom it was present; however, the onset of unipolar illness in the adopting parents occurred, in every case, after the onset of manic-depressive illness in their children, whereas in the biological parents, onset of unipolar illness occurred almost always previous to the onset in their children. This observation strengthens the genetic hypothesis by suggesting that the adoptive parents' unipolar illness might be more reactive and less severe than that of their biological counterparts; early onset is often considered to be an index of severity in psychiatric disorder.

In summary, we found a greater degree of psychopathology, particularly of affective illness, in parents genetically related to manic-depressive probands than in parents who adopted and raised these same individuals. The results demonstrate the importance of genetic factors in the aetiology of manic-depressive illness.

We thank Judith Klotz for assistance and Professor E. Sand and Dr P. Linkowski for their contribution to the study. This work was supported by the New York State Department of Mental Hygiene and by a grant from the University of Brussels.

JULIEN MENDLEWICZ

Psychiatric Institute,
University of Brussels,
Brugmann Hospital,
4, Place Van Gehuchten,
1020 Brussels, Belgium

JOHN D. RAINER

New York State Psychiatric
Institute and Columbia University,
New York, New York 10032

Received 15 April; accepted 30 May 1977.

- Zerbin-Rudin, E. in *Humangenetik* 2 (ed. Becker, P. E.) 446-577 (Georg Thieme, Stuttgart, 1967).
- Winokur, G., Clayton, P. J. & Reich, T. *Manic-Depressive Illness* (C. V. Mosby, St Louis, 1969).
- Mendlewicz, J. & Rainer, J. D. *Am. J. hum. Genet.* 26, 692-701 (1974).
- Mendlewicz, J. & Fleiss, J. L. *Biol. Psychiat.* 9, 261-294 (1974).
- Heston, L. L. *Br. J. Psychiat.* 112, 819-825 (1966).
- Wender, P. H., Rosenthal, D. & Kety, S. S. in *The Transmission of Schizophrenia* (eds Rosenthal, D. & Kety, S. S.) 235-250 (Pergamon, Oxford, 1968).
- Kety, S. A., Rosenthal, D. & Wender, P. H. in *The Transmission of Schizophrenia* (eds Rosenthal, D. & Kety, S. S.) 345-362 (Pergamon, Oxford, 1968).
- Rosenthal, D., Wender, P. H., Kety, S. S., Welner, J. & Schulsinger, F. *Am. J. Psychiat.* 128, 307-311 (1971).
- Schulsinger, F. *Int. J. ment. Hlth* 1, 190-206 (1972).
- Goodwin, D. W., Schulsinger, F., Hermansen, L., Guze, S. & Winokur, G. *Archs gen. Psychiat.* 28, 238-248 (1973).
- Spitzer, R. L., Endicott, J. & Fleiss, J. L. *Compr. Psychiat.* 8, 321-343 (1967).
- Feighner, J. P. et al. *Archs gen. Psychiat.* 26, 57-63 (1972).
- Hollingshead, A. B. & Redlich, F. C. *Social Class and Mental Illness* (Wiley, New York, 1968).
- Mendlewicz, J. in *The Impact of Biology on Modern Psychiatry* (eds Gershon, E. S., Belmaker, R. H., Kety, S. S. & Rosenbaum, M.) 229-240 (Plenum, New York, 1976).
- Baron, M. *Archs gen. Psychiat.* (in the press).

Number of trophic levels in ecological communities

ECOLOGICAL food chains are typically short, consisting of not more than four or five trophic levels. This is usually explained by a reduction in the energy which is available to successive links in the food chain^{1,2}. In contrast, we believe that the number of trophic levels is constrained by population dynamics and not by ecological energetics.

In the extreme, food chains must obviously be limited by the flow of energy. Slobodkin's³ hypothetical predator occupying a trophic level higher than twenty and needing a continent to support it is a dramatic example. However, if food chains are primarily limited by energy they should be longer in more productive ecosystems; for example with an ecological efficiency² of approximately 10%, trophic levels should increase at the rate of one for each tenfold increase in the energy entering the system. This does not seem to be true. Primary productivity ranges over at least four orders of magnitude in terrestrial ecosystems, over three for aquatic systems³; yields of fish and the productivity of animal populations range over five or more orders of magnitude^{4,5}; yet the number of trophic levels supported by these particular plant and animal populations does not seem to be closely correlated with the productivity of the base of the food chain. Food chains are not noticeably shorter in barren Arctic and Antarctic terrestrial ecosystems^{6,7} compared with a productive tropical savannah⁸ or the fish guilds of a tropical coral reef⁹.

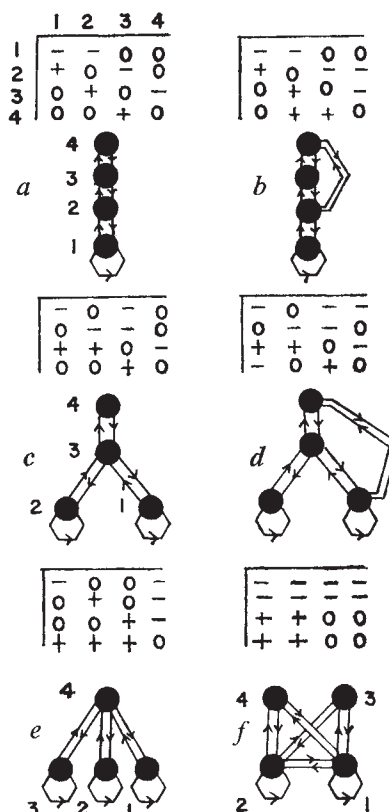
One explanation for short food chains is that it is advantageous to feed low in the chain, where more food is available. The effects of body size¹, or the seasonality and predictability of resources may also be important^{10,11}. However, the dynamically fragile nature of long food chains does not seem to have been discussed.

Consider a system of n interacting species described by equations of the form

$$\frac{dX_i}{dt} = X_i(b_i + \sum_{j=1}^n a_{ij}X_j) \equiv F_i \quad (1)$$

where X_i is the population size and b_i the instantaneous rate of growth of the i th species, and a_{ij} is the *per capita* effect of the

Fig. 1 The matrices show whether the species along each row has a positive (+), negative (−) or no (0) effect on the species in each column at equilibrium; the figures below indicate these relationships diagrammatically.



j th species on the i th species. We used equation (1) to construct model food chains of varying length and complexity, and judged their feasibility by whether or not they were locally stable, and if they were, how long they took to return to equilibrium after a disturbance (the 'return time'). Long return times imply an increased probability of extinction in the real world¹²⁻¹⁴. The dynamics of equation (1) near equilibrium are determined by the eigenvalues of a matrix which is the Jacobian

$$\partial F_{ij} = 1, n$$

$$\partial X_{ji} = 1, n$$

evaluated at equilibrium. A system which contains non-negative eigenvalues will be locally unstable, while the return time of a locally stable system will be roughly proportional to the reciprocal of the largest eigenvalue. Formally

$$\text{Return time} = -1/\text{Real}(\lambda_{\max}) \text{ (for all } \lambda < 0 \text{)}$$

In general, as the number of species in a model ecosystem increases, the probability of local stability decreases¹⁵. But it is possible using differential equations to construct linear food chains of any length that are locally stable (Fig. 1a), and Maynard Smith¹⁶ has argued that "the lengthening of food chains will not by itself cause instability". This view gives a distorted impression of what is biologically feasible, because as the number of trophic levels increases, so do return times.

We have followed established practice^{12,17-19}, and investigated eigenvalues of matrices whose elements are random variables subject to constraints of sign and absolute magnitude. The possibilities investigated are shown in Fig. 1. Parameter values were set by the biological details implied by the model¹³. The diagonal elements of the Jacobian are $X_i^* a_{ii} = -b_i X_i^*/K_i$ where X_i^* is the equilibrium population of the i th species, and K_i its equilibrium in the absence of all other species. Since the X_i^* cannot exceed K_i these values will be in the interval $(0, -b_i)$ (the b_i were set equal to 1 in each species). The off diagonal elements are also of the form $X_i^* a_{ij}$ where the sign of a_{ij} will depend on whether i is feeding on j or vice versa, or whether i and j are competitors. For predator-prey interactions (Fig. 1 a-e) a_{ij} is the number of new predators produced per prey eaten; the latter will usually be much larger than the former. The X^* will also differ, since predators are likely to be fewer than their prey at equilibrium. The effects of the predator on the prey population were therefore uniformly distributed on the interval $(0, -10)$ and the effects of the prey on the predator on the interval $(0, +0.1)$. For competition (Fig. 1f) $X_i^* a_{ij}$ is the magnitude of the interspecific competition. This will usually be less than the absolute value of the intraspecific competition ($X_i^* a_{ii}$) and was uniformly distributed on the interval $(-X_i^* a_{ii}, 0)$. For each of the six configurations of signs shown in Fig. 1 nearly 2,000 matrices were created whose elements satisfied these general constraints. Figure 2 shows their return times. (Results were qualitatively the same in 1,000 initial simulations in which the matrix elements had absolute values between zero and one, suggesting that they are not sensitive to the assumptions about the magnitude of the matrix elements.)

Consider first the models without omnivores (Fig. 2a, c, e). As the number of trophic levels increases, so do return times. For two trophic levels, nearly 50% of the models had return times less than or equal to five; with three trophic levels, this drops to 5% and with four, to less than 1%. Figure 2 also shows the percentage of models where return times exceed 150. With two trophic levels this is very small (0.1%), but is 9% and 34% respectively with three and four trophic levels.

A two-species system with one predator and one prey can be examined analytically. Here there are as many species as trophic levels, analogous to model (a) of Figs 1 and 2. About 70% of the two-species models had return times less than or equal to five—a considerably larger percentage than is found in model (a), but comparable with the situation in model (e). Hence, models with different numbers of species and the same

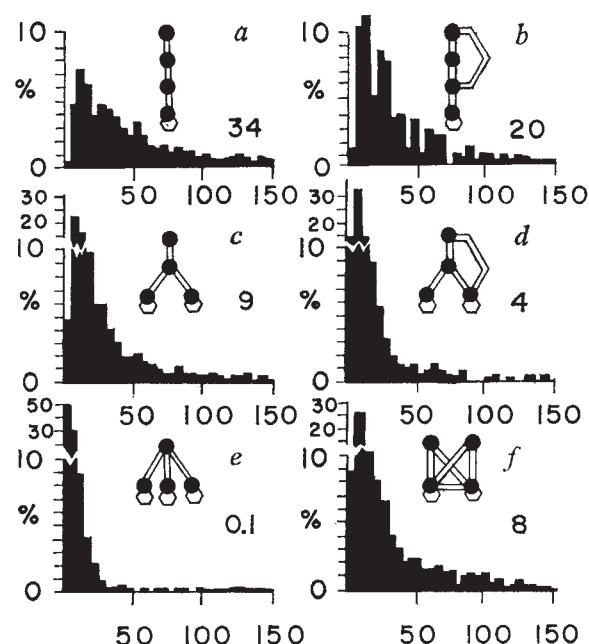


Fig. 2 The distribution of the reciprocals of the largest eigenvalues (a measure of return times) from nearly 2,000 matrices of each configuration. The number on each histogram is the percentage of those reciprocals which have a value greater than 150.

number of levels, and models with the same number of species but different numbers of levels lead to similar conclusions. We suggest that this increase in return times with an increasing number of trophic levels will play an important part in limiting the lengths of food chains subject to the perturbations of the real world.

With omnivores, 78% of both the three (Fig. 1d) and four (Fig. 1b) trophic-level models are unstable. Return times in the remaining 22% are illustrated in Fig. 2b and d, which show that omnivores do not affect the conclusion that longer food chains imply longer return times; however, the distributions are shifted slightly towards faster values. Whether or not the addition of omnivores (further complexity) contributes to the 'stability' of a system then depends on how these results are evaluated. For systems assembled at random, omnivory increases the probability of instability; however those that are locally stable have faster return times, and in this sense are more stable. This paradox does not seem to have been mentioned in studies on 'complexity' and 'stability' in model ecosystems.

Figure 2f includes competitive effects in the lowest trophic level. As expected²⁰, 53% of the models were unstable but the return times of the remainder were no greater than in model (c), a three-trophic-level model, which lacks the destabilising factor of competition. However, it is known that a 'pure' competition community (one trophic level) of n interacting species may have more than one locally stable equilibrium²¹. It is not clear how the addition of higher trophic levels would influence the existence of, and rates of return to alternative equilibria; further work is required on this point.

We have only considered one type of model (differential equations) and one aspect of their dynamic behaviour (return time). It is easy to construct very different models, implying different biological details, and with very different behaviours. It is therefore encouraging to note that in a discrete generation (difference equation) model of an insect host-parasitoid interaction (two trophic levels), large areas of stable parameter space disappear when a third trophic level (a hyperparasite) is added²². This reinforces our view that the number of trophic

levels in ecological communities is determined not by ecological energetics, but by population dynamics.

We thank John Beddington, Charles Free, Robert May and Stuart McNeill for helpful discussion.

S. L. PIMM

Department of Biological Sciences,
Texas Tech University,
Lubbock, Texas 79409

J. H. LAWTON

Department of Biology,
University of York,
Heslington, York, UK

Received 25 March; accepted 17 May 1977.

- ¹ Hutchinson, G. E. *Am. Nat.* **93**, 145–159 (1959).
- ² Slobodkin, L. B. *Growth and Regulation of Animal Populations* (Holt, Rinehart and Winston, New York, 1961).
- ³ Whittaker, R. H. *Communities and Ecosystems* (Collier Macmillan, London, 1975).
- ⁴ Mann, K. H. *J. Anim. Ecol.* **34**, 253–275 (1965).
- ⁵ McNeill, S. & Lawton, J. H. *Nature* **225**, 472–474 (1970).
- ⁶ Elton, C. S. *Animal Ecology* (Sidgwick and Jackson, London, 1927).
- ⁷ Strong, J. *Antarctic Res. Ser.* **10**, 357–371 (1967).
- ⁸ Lamotte, M. in *Tropical Ecological Systems* (eds Golley, F. B. & Medina, E.) (Springer-Verlag, Berlin, 1975).
- ⁹ Goldman, B. & Talbot, F. H. in *Biology and Geology of Coral Reefs*. 3 (eds Jones, O. A. and Endean, R.) (Academic, New York, 1976).
- ¹⁰ Menge, B. A. & Sutherland, J. P. *Am. Nat.* **110**, 351–369 (1976).
- ¹¹ Janzen, D. in *Evolutionary Strategies of Parasitic Insects and Mites* (ed. Price, P. W.) (Plenum, New York, 1975).
- ¹² Levins, R. in *Towards a Theoretical Biology*, Vol. 3. (ed. Waddington, C. H.) (Edinburgh University Press, 1970).
- ¹³ Levins, R. *Evolution in Changing Environments* (Princeton University Press, 1968).
- ¹⁴ Beddington, J. R., Free, C. A. & Lawton, J. H. *J. Anim. Ecol.* **45**, 791–816 (1976).
- ¹⁵ May, R. M. *Stability and Complexity in Model Ecosystems* (Princeton University Press, 1973).
- ¹⁶ Maynard Smith, J. *Models in Ecology* (Cambridge University Press, 1974).
- ¹⁷ May, R. M. *Nature* **238**, 413–414 (1972).
- ¹⁸ De Angelis, D. L. *Ecology* **56**, 238–243 (1975).
- ¹⁹ Gilpin, M. E. *Nature* **254**, 137–139 (1975).
- ²⁰ May, R. M. *Ecology* **54**, 638–641 (1973).
- ²¹ Gilpin, M. E. & Case, T. J. *Nature*, **261**, 40–42 (1976).
- ²² Beddington, J. R. & Hammond, P. *J. Anim. Ecol.* (in the press).

Large benthic microbial communities in sulphide biota under Peru–Chile Subsurface Countercurrent

BENTHIC observations off the coast of Chile have consistently disclosed the presence of large coherent microbial communities living at depths of about 50–280 m in the H₂S-containing sediments of the shelf in contact with the deoxygenated waters of the Peru–Chile Subsurface Countercurrent (SCC). Similar observations were also made off Peru in 1969 by Gilbert T. Rowe, and in 1976 by G. T. Rowe and John Waterbury of Woods Hole Oceanographic Institution. The microflora, which has only been reported once before in the literature¹, has been known for years by the local fishermen who call them *estopa* (Spanish for uncleaned wool or flax) due to the filamentous appearance of its main components. In this report I describe this massive microbial community which includes organisms typical for sulphide biota, and may have unsuspected importance in the ecology and economy of the sea off western South America.

Repetitive quantitative grab sampling off Concepcion, Chile (36°35'30"S, 73°04'20"W) at 60-m depth, indicated biomass of 106 g (wet weight) per 0.1 m² for the microbial component, while the benthic infaunal biomass in the same sample attained only 11.5 g (wet weight) per 0.1 m² (Peterson 0.1-m² sample washed through a 0.25-mm² sieve). Preliminary examination of the community has shown that it consists of many kinds of prokaryotes. The main constituent, however, is typically large filaments of *Thioploca* spp. This genus of gliding bacteria has not been previously recorded in open oceans. The *Thioploca* spp. fall clearly into three groups according to their cell diameter: (1) 30–40 µm, (2) 15–20 µm, and (3) 2.5–5 µm, (Dr Maier, Ohio University, personal communication). Other members of the community are *Oscillatoria*-type blue-green algae (cyanobac-

teria), flexibacteria (probably of the genus *Chlorophlexis*) and various other forms of bacteria.

The *Thioploca*-like forms appear as many individual filaments within a common sheath (Fig. 1, inset) forming large, whitish-yellow, twine-like structures that attain 100–500 µm in diameter and several cm in length, therefore perfectly visible to the naked eye (Fig. 1). Off the Bay of Concepcion, where most of the recent observations have been carried out, the sediments consist of a loose superficial yellowish-brown layer of a few mm thick, an intermediate layer of black H₂S-containing sediment about 8 cm thick, and a lower dark-brown layer of sticky compacted mud¹. The filamentous microbial material occurs in the upper two layers, giving the sediment a soft, spongy texture. The sediment, a diatomaceous mud, also contains large quantities of assorted organic debris such as fish scales, fish bones, faecal pellets, few shell fragments, empty polychaete tubes and some terrestrial vegetal remains.

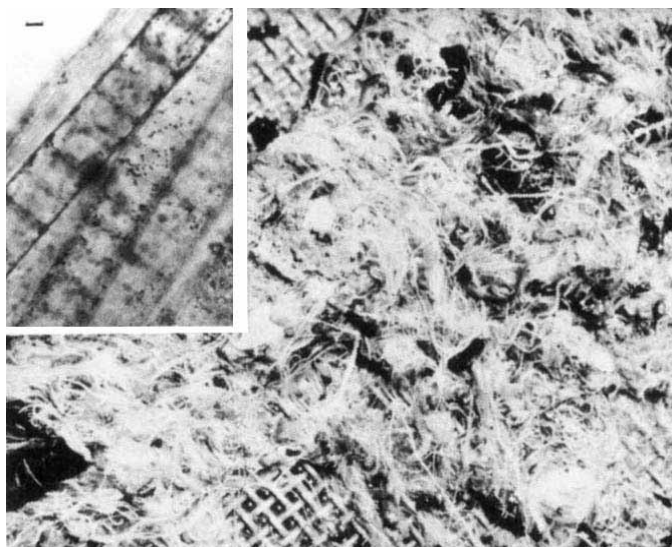
Although the genus *Thioploca* is classified among the colourless Beggiatoaceae², some species of the genus contain a greenish blue pigment and have therefore an uncertain taxonomic relationship to the *Beggiatoa*³. Ethanol extracts from seawater-washed filaments have given absorption spectra typical for chlorophyll *a*, with major peaks at about 410 nm and 670 nm; however, it is probable that this pigment is associated with oscillotian cyanobacteria known to be components of this community. The gliding of independent filaments occurs within their common sheath at speeds of up to 12.5–25 µm min⁻¹.

Whatever the taxonomic position of these *Thioploca*-like forms, their large standing-crop suggests an important role in the ecology of the upwelling biome off southwestern South America.

The SCC, a wedge-shaped water mass distributed between northern Peru and southern Chile (to about 41°S), is characterised by relatively high salinity and temperature, and extremely low oxygen content, typically under 0.1 ml O₂ per l. Nutrient concentrations are high^{4–6}.

The presence of filamentous microbial associations should be looked for in other parts of the world where similar oceanographic conditions exist. Recently a single *Thioploca*-like filament was observed in a small sediment sample collected off Walvis Bay in

Fig. 1 Filamentous microflora deposited on a 1-mm² mesh 12-inch diameter geological sieve after washing a 0.1 m² Petersen grab sediment sample collected at 100-m depth off the Bay of Concepcion, central Chile, October 7 1975. At 90 m salinity was 34.54‰, temperature 9.71 °C, and dissolved oxygen 0.54 ml l⁻¹. Inset, photomicrograph of portion of *Thioploca*-like form no. 1. The common sheath and four cell-chains containing intracellular sulphur granules are visible. Cell dimensions are 2436 µm diameter and 3036 µm length. Bar represents 10 µm.



south-western Africa (J. W. Farrington). In this upwelling region a mysterious benthic 'slimy grass' has been reported at depths of about 90–130 m (ref. 7), occurring with a low-oxygenated sub-surface countercurrent impinging against the shelf⁸.

The coincidence of the depth distribution of the microbial community off Chile and that of the principal shrimp (both penaeid and galatheid) and hake fishing grounds⁹, strongly suggests a possible trophic relationship in view of the fact that most of the standing-crop therein seems to be made up of filamentous bacteria.

This work was sponsored by an OIP Grant at the Woods Hole Oceanographic Institution, ABC Foundation and by the NOAA.

VICTOR A. GALLARDO

Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts 02543, and
Departamento de Biología Marina y Oceanografía,
Universidad de Concepción,
Concepción, Chile

Received 28 June 1976; accepted 1 February 1977.

- ¹ Gallardo, V. A. *Gayana (Zool.)* **10**, 3–15 (1963).
- ² Leadbetter, E. R. in *Bergey's Manual of Determinative Bacteriology* (eds Cowan, S. T. et al.), 112–113 (Williams and Wilkins, Baltimore, 1974).
- ³ Maier, S. *Ibid.*, 115–116.
- ⁴ Brandhorst, W. *Ber. Landw. Hamburg* **43**, 148–187 (1967).
- ⁵ Hartmann-Schroeder, G. & Hartmann, G. *Mitt. Hamb. zool. Mus. Inst.* **62**, 1–384 (1954).
- ⁶ Wooster, W. S. & Gilmartin, J. J. *mar. Res.* **19**, 97–100 (1961).
- ⁷ Bonde, C. von *Rep. Fish. Mar. Biol. Surv. Un. S. Afr.* **5**, 10–85 (1928).
- ⁸ Decker, A. H. B. de *Invest. Rep. Div. Fish. Un. S. Afr.* **84**, 1–24 (1970).
- ⁹ Ledermann, J. *Infmes. Pesq. Inst. Fom. Pesq. Santiago, Chile.* **57**, 1–10 (1975).

Drinking behaviour induced by intracranial injections of eleodoisin and substance P in the pigeon

THE only peptide known which rapidly and consistently elicits a specific behavioural response when injected into the brain is angiotensin II, which in picomole doses causes reptiles, birds and mammals to seek out and drink water¹. But, there is evidence to suggest that other peptides such as substance P may also participate in the control of brain function and behaviour. Substance P is distributed throughout the mammalian peripheral and central nervous system, particularly in myenteric plexus, sensory afferent pathways, hypothalamus and substantia nigra². In the human brain regional concentrations as high as 0.5×10^{-9} mol per g wet tissue have been reported³. Substance P is a member of a family of peptides with similar pharmacological properties and which also includes eleodoisin, an undecapeptide obtained from the salivary glands of certain Mediterranean cephalopods⁴. These peptides share a similar amino acid sequence at the C-terminal end (Fig. 1). Both eleodoisin and substance P stimulate nerve cell activity in vertebrates and invertebrates and may act as regulators of neural activity⁵, but no specific behavioural effect of these peptides has yet been reported. We report here that eleodoisin and substance P elicit vigorous drinking when injected into the brain of conscious pigeons.

At a preliminary operation intracranial cannulae (outside diameter (o.d.) 0.6 mm) were implanted stereotactically in male and female pigeons (*Columba livia*, 300–450 g) under Equithesin anaesthesia (3.0 ml per kg body weight). The cannulae terminated in the lateral or third ventricle, preoptic area, lateral hypothalamus or striatum. The pigeons were allowed at least one week to recover from the operation before testing began. The injections were made in conscious birds through stainless steel tubing (o.d. 0.3 mm) inserted into the implanted cannulae and projecting 1 mm beyond the cannula tip into the brain tissue. All drugs were dissolved in 0.9% saline and injected in volumes of 1 μ l over 10 s. The highest dose of eleodoisin was injected in 10 μ l because of its low solubility. The

pigeons were returned to their home cages immediately after injection where food, water and mineral grit were available. Only one drug was tested per day, with at least a 2-d rest between tests. The order of testing was random. The drugs injected were eleodoisin (Farmitalia), eleodoisin-related peptide, substance P, Asp¹Ile²-angiotensin II, arginine vasotocin (all from Bachem), vasopressin (Pitressin, Parke-Davis), bradykinin (Sigma) and L-glutamate (Sigma).

Intracranial injections of as little as 10^{-11} mol eleodoisin consistently caused pigeons to drink water. The drinking was vigorous but completely normal, and usually began within 1 or 2 min after injection. The mean latency ($\pm$ s.e.m.) in 13 pigeons in response to 10^{-10} mol of eleodoisin was 1.5 ± 0.2 min. The amount of water drunk was dose dependent and was large (Fig. 2). Intracranial injection of 10^{-9} mol eleodoisin caused pigeons to drink more than they would normally ingest during the entire day, usually in a period of less than 5 min. The response was repeatable from day to day and always specific to drinking. None of the birds ever ate or engaged in any other behaviour consistently after injection. When the water spouts were removed from the cage the pigeons repeatedly looked around the cage for water and pecked frequently at the hole where the water spout normally projected. Immediately the spout was replaced they drank vigorously. When drinking was prevented eleodoisin did not cause increased body water losses, as measured by changes in body weight, and there were no changes in cloacal temperature. Intravenous injections of 10^{-10} or 10^{-9} mol of eleodoisin did not cause pigeons to drink ($N = 5$), indicating that the effect of intracranial injections was not due to leakage from the brain.

Since this drinking response was identical to that which we observed following intracranial injections of angiotensin II in the pigeon⁶, we compared the relative potencies of these two peptides. On a molecule per molecule basis eleodoisin was only about one order of magnitude less potent than angiotensin II (Fig. 2). Thus in the pigeon eleodoisin is as active a dipsogen as carbachol is in the rat¹.

The C-terminal hexapeptide fragment of eleodoisin (eleodoisin-related peptide) and substance P also caused pigeons to drink when injected into the brain. These peptides were less active than eleodoisin, but again the response was specific to drinking and the amount of water drunk was significantly greater than that following control injections of 0.9% saline (Fig. 2). On the other hand, intracranial injections of vasopressin (10^{-12} , 10^{-11} mol), vasotocin (10^{-11} , 10^{-9} mol), bradykinin (10^{-10} , 10^{-9} mol) or L-glutamate (10^{-10} , 10^{-7} mol) had no significant effects.

Eleodoisin caused drinking in all brain regions sensitive to the dipsogenic actions of angiotensin II. These included the lateral ventricle ($N = 3$), third ventricle ($N = 3$), preoptic region ($N = 6$) and lateral hypothalamus ($N = 4$). There were no significant differences in the response to eleodoisin from these four areas but intakes tended to be highest following preoptic or third ventricular injection. Two pigeons with cannulae in the neostriatum which did not pass through the lateral ventricle failed to drink to

Fig. 1 Amino acid sequences of peptides that stimulate drinking when injected into the brain of the pigeon.

Angiotensin II	Asp - Arg - Val - Tyr - Ile - His - Pro - Phe
Eleodoisin	Pyr - Pro - Ser - Lys - Asp - Ala - Phe - Ile - Gly - Leu - Met (NH ₂)
Eleodoisin Related Peptide	H ₂ N - Lys - Phe - Ile - Gly - Leu - Met (NH ₂)
Substance P	Arg - Pro - Lys - Pro - Gln - Gln - Phe - Phe - Gly - Leu - Met (NH ₂)

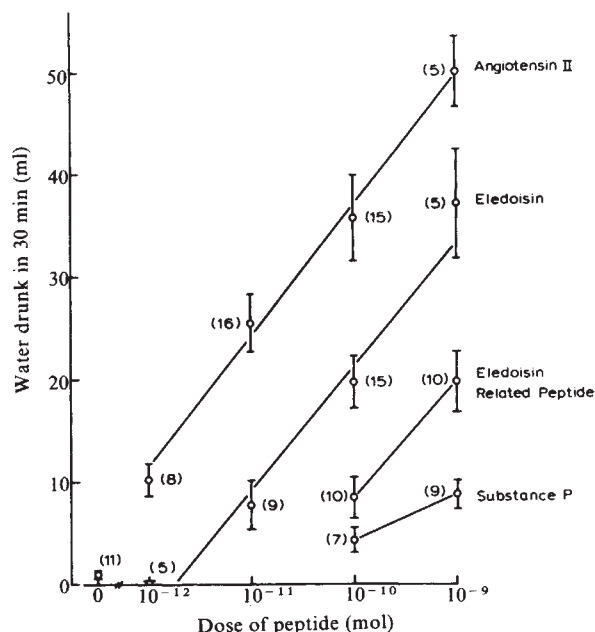


Fig. 2 Mean amounts ($\pm$ s.e.m.) of water drunk in 30 min (no. of birds tested in parentheses) following intracranial injection of different peptides. Controls received 1 μ l 0.9% NaCl. Regression lines are shown for angiotensin II and eledoisin.

eledoisin or angiotensin II. One pigeon with a striatal cannula which did pass through the lateral ventricle drank to injections of the peptides, probably because of reflux of the injected material into the ventricular system as has been shown for angiotensin II injections in the rat⁷.

The similar time course and dose-response relationship of drinking following intracranial injections of angiotensin and eledoisin in the pigeon and the similar neuro-anatomical distribution of sensitive brain sites suggest a similar or common mechanism. It is unlikely, however, that these two peptides are activating the same receptor site, because eledoisin is structurally so very different from angiotensin II and because we have found that the receptor sites for angiotensin-induced drinking in rats and pigeons are highly specific for the angiotensin II octapeptide. Only slight modifications or removal of amino acids in the angiotensin peptide chain, particularly at the C-terminal end, severely reduce or even abolish activity altogether^{8,9}. Other peptides such as vasopressin, vasotocin or bradykinin all fail to stimulate drinking in the pigeon.

Eledoisin and substance P are potent stimulants of neuronal activity¹⁰, but a nonspecific activation of nervous tissue cannot explain the consistent and specific behavioural response observed here. Drinking was elicited by injection of these substances into several different brain regions which also contain the neural elements of many other behavioural functions. Furthermore, intracranial injections of glutamate, another potent excitatory substance, did not cause pigeons to drink.

This is the first demonstration that eledoisin and substance P can elicit a specific and complete behavioural response when injected into the brain. What is surprising is that this response should be identical to that elicited by angiotensin II, the only other peptide known to have such a pronounced behavioural effect. Angiotensin II is a potent vertebrate hormone that is released in response to extracellular fluid losses and has many actions which contribute to the restoration of body fluid balance¹¹. The significance of the drinking response to eledoisin and

substance P in terms of body fluid regulation remains to be determined.

This work was supported by the MRC and the MRC of Canada.

M. D. EVERED
J. T. FITZSIMONS
G. DE CARO

The Physiological Laboratory,
University of Cambridge, UK

Received 9 May; accepted 31 May 1977.

- 1 Fitzsimons, J. T. *Physiol. Rev.* **52**, 468–561 (1972).
- 2 Leeman, S. E. & Mroz, E. A. *Life Sci.* **15**, 2033–2044 (1974).
- 3 Powell, D., Leeman, S., Tregear, G. W., Niall, H. D. & Potts, J. T., Jr *Nature new Biol.* **241**, 252–254 (1973).
- 4 Erspamer, V. & Anastasi, A. *Experientia* **18**, 58–59 (1962).
- 5 Henry, J. L., Krnjevic, K. & Morris, M. E. *Can. J. Physiol. Pharmacol.* **53**, 423–432 (1975).
- 6 Evered, M. D. & Fitzsimons, J. T. *J. Physiol., Lond.* **263**, 193–194P (1976).
- 7 Johnson, A. K. & Epstein, A. N. *Brain Res.* **86**, 399–418 (1975).
- 8 Fitzsimons, J. T. *J. Physiol., Lond.* **214**, 295–303 (1971).
- 9 Evered, M. D. & Fitzsimons, J. T. *J. Physiol., Lond.* **263**, 252–253P (1976).
- 10 Barker, J. L. *Physiol. Rev.* **56**, 435–452 (1976).
- 11 Fitzsimons, J. T. *Kidney Int.* **10**, 3–11 (1976).

Cells selective to binocular disparity in the cortex of newborn lambs

In the nineteenth century there was a vigorous controversy over the role of visual experience in the development of stereoscopic depth perception^{1,2}. Recent neurophysiological findings suggest that certain cells in the visual cortex of cats^{3,4}, monkeys⁵ and sheep⁶ may provide the neural basis for stereopsis. Each cell is driven optimally by a stimulus at a certain distance from the animal, and different cells prefer different retinal disparities corresponding to objects at various distances. In cats, the neural pathways from the two eyes to individual cortical cells are labile and may be modified by visual deprivation during the first few weeks of life^{7–10}. The visual cortex of the newborn kitten is immature and disparity selectivity appears only after exposure to a normal visual environment¹¹. We find, however, that neither of these two conclusions is true of lambs.

It has been suggested that the neural mechanisms underlying stereopsis require visual experience for their development^{11–15}. 'Plasticity' of binocular correspondence was observed by Schlaer¹⁶ and was thought to account for the preservation of correspondence in spite of changing interocular geometry during development¹⁴. Newborn animals (including lambs) will avoid a 'visual cliff'^{17,18}, but it is unclear whether stereopsis is involved, particularly since chicks and rats will continue to avoid the cliff even with one eye closed¹⁷. Good depth judgment would be of obvious survival value in the wild, where a 1-d-old mountain lamb will avoid a ravine¹⁹.

We have begun to investigate the possible role of visual experience in the development of stereopsis by recording from the visual cortex of lambs. In adult sheep (and goats) a substantial number of cells, especially in V₂, can be driven only by a binocular stimulus at a particular disparity. In V₂, the optimal vertical disparities are close to zero but a wide range of horizontal disparities (about $\pm 4^\circ$) occurs⁶. Our strategy was to look for such depth-sensitive cells in the visual cortex of newborn lambs which have had no visual experience. Lambs are born robust and with clear optic media, which makes them very suitable for studying the development of disparity detecting mechanisms, especially since large horizontal disparities occur in the adult.

Six animals (five newborn lambs and one newborn kid) were used. Three were born and kept in complete darkness, and recorded from on the first, fifth and sixth days after birth. The other three were born in the light (albeit moonlight), kept in normal lighting, and recorded from within 21 h of birth. They were anaesthetised initially with 2% halothane in oxygen and later maintained on intravenous

pentobarbitone (0.5 mg per kg body weight per h) and a N_2O/O_2 mixture (60%, 40%). This dosage was sufficient to give full surgical anaesthesia in newborn lambs, and larger doses caused respiratory arrest. Tubocurarine (0.1 mg per kg per h) and gallamine triethiodide (3 mg per kg per h) were used as paralytics to reduce eye movements. (For details see ref. 6.)

Tungsten-in-glass microelectrodes with impedances of 1–5 M Ω at 1,000 Hz were used²⁰. Visual stimuli were usually hand-held targets moved against a screen 90 cm from the eyes and the separate monocular receptive fields were drawn on a tangent screen. For most of the units we also tested the effect of varying the retinal disparity by means of a Risley prism in front of the left eye. Units and background activity were tested once every 100 μ M or so of electrode descent.

The positions of the eyes were monitored using a Fison indirect ophthalmoscope. The projections of the optic disk and vertical retinal artery were plotted on extensions of the tangent screen. Hence eye movements greater than 0.5° could be detected and corrected for.

Although the visual mapping on the cortex sufficed to distinguish V_1 and V_2 , we identified the needle tracks histologically. Of the 231 units whose responses we tested, 30 were in V_1 , 192 were in V_2 , and 9 were not reliably located: 119 of the V_2 units were from lambs with no visual experience whatsoever. Here we describe only the units in V_2 .

The results were clear-cut. Almost all the units were visually responsive, and more than 95% were sharply tuned to orientation. There was no noticeable difference in the proportion of tuned cells or in their sharpness of tuning compared with the adult. Most of the units responded much better to a moving oriented bar than to a moving spot and those which would respond to a flashed bar were selective to its orientation. In this sense they were truly orientation selective rather than direction selective cells. Also the cells seemed to be arranged in 'orientation columns' and systematic changes in orientation could be seen as the electrode crossed several columns (compare with baby monkey²²). Figure 1 illustrates one such 'orientation

Fig. 1 An orientation sequence. The optimal orientations of 13 single units are plotted at the depth they were recorded in a penetration through V_2 . The only other unit recorded was not clearly orientated, and its position is marked by a filled circle. Clearly, the optimal orientation changed monotonically as the electrode advanced through the cortex, which suggests that the systematic organisation of orientation columns which occurs in the adult was already present. This lamb was born in complete darkness a few hours before the experiment, and its eyes were first exposed to light a few minutes before the start of this, the first penetration.

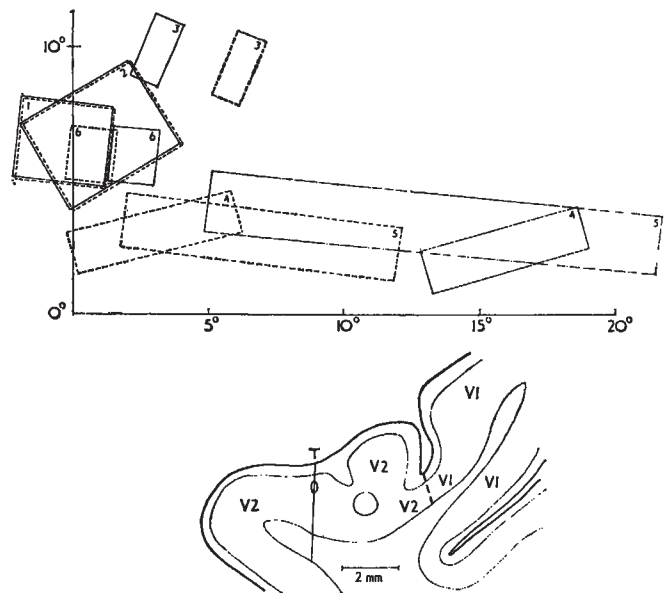
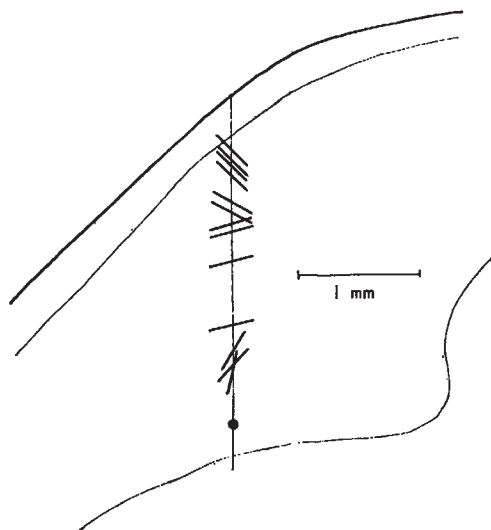


Fig. 2 The monocular receptive fields, through each eye, of the single units recorded in a penetration through V_2 , illustrating the occurrence of binocular disparities. This particular lamb was born 21 h before the start of the experiment, but it had less than 12 h of visual experience. Comparable results were obtained in a lamb with no visual experience whatsoever. The zero vertical meridia of the two eyes have been aligned, so the receptive fields of zero disparity cells are exactly superposed. The fields of the left eye are shown by discontinuous lines, and the numbers indicate the order in which the units were recorded. It can be seen that the disparities were purely horizontal and spanned a very large range on account of two units with unusually large (up to 13°) uncrossed disparities. We confirmed these disparities by tracking back and down again with the electrode, and we checked the eye positions after each large change of disparity. Also, the orientations of the receptive fields are the same for the two eyes. The inset shows the position of the electrode track (T), including a lesion, in V_2 . The V_1/V_2 border has been estimated by comparing the section with one 1 mm posterior in which the site where the visual map reversed was found with five electrode penetration.

sequence' from the very first penetration made in one of our newborn lambs. The orientations of the receptive fields of individual binocular neurones were determined separately through each eye, and were found to be precisely matched. Thus, in lambs, at least, there is no basis for the suggestion that the alignment of the optimal orientations in the two eyes requires visual experience^{15,21}.

The receptive field properties did not differ noticeably from those in adults, and simple, complex and hypercomplex cells could all be found (as in the cortex of newborn monkeys²²). There was one exception, however: 16 units were found for which the response was excitatory from one eye and strongly inhibitory from the other, with greatest inhibition at zero disparity. We have not so far found such units in adult sheep. The separate monocular fields of binocular neurones could be mapped on the tangent screen, and variations in binocular disparity (that is, the range of 'scatter' of receptive field disparities) could be precisely determined (Fig. 2). The most frequent disparity was taken to be zero. In three of the animals there was clear evidence of horizontal disparities of up to $\pm 4^\circ$, but in one animal there were two cells with exceptionally large disparities (Fig. 2). The range of vertical disparities was close to zero in all six animals. Disparity 'tuning' curves could be obtained for many of the cells by varying the strength of the prism in front of the left eye. The peak of the tuning curve usually corresponded to what one would expect from actually measuring the angular distance between the centres of the monocular receptive fields. Hence

cells selectively 'tuned' to a large range of non-zero disparities, as well as zero, were observed. These results cannot have been seriously contaminated by eye movements, since the positions of the eyes were monitored to within 0.5° , and the disparities were much larger than this. Furthermore, on some occasions we withdrew the electrode and drove it down the track again, and the same disparities were found at the same depths.

There were interesting differences between the V_2 binocular neurones of newborn lambs and those seen in the adults (more than three months). In adults most of the neurones give a strongly facilitated response to a binocular stimulus at the optimal disparity and many are true 'and-gates', not having discernable monocular receptive fields. In newborn lambs, however, all the binocular neurones could be independently driven through either eye and the degree of binocular facilitation or 'synergy' was considerably less than in the adult. The binocular facilitation in a normal seven-week-old lamb in which we recorded from 36 units was intermediate between that in the adult and in the newborn lamb. Whether the conversion of immature binocular neurones into true 'and-gates' requires visual experience, remains to be determined. The appearance of additional inhibitory mechanisms may be required for this.

In visually naive kittens, Pettigrew¹¹ found that the neurones were not selective to disparity and yet showed binocular facilitation at least as great as in the adult. This is the opposite of what we have found in lambs, where many of the cells were tuned to disparity, but the binocular enhancement was substantially less than in the adult. While several authors^{9,11,14-16} have suggested that visual experience may have an active role in organising the feature-detecting properties of cortical neurones, there seems little need for this in the lamb, for we find that in virtually all the cells in V_2 , the following are all specified by a genetic mechanism: (1) orientation selectivity; (2) organised 'orientation columns' and systematic changes in orientation tuning (orientation 'sequences'); (3) interocular matching of orientation selectivity, and (4) 'hypercomplex' type receptive field organisation. Most importantly, we have found that disparity selectivity is present at birth. This confirms Hering's theory¹ that the establishment of correspondence between disparate retinal areas does not require visual experience, although the progressive increase in the degree of binocular facilitation may turn out to require visual experience.

We thank Mrs Pat Donaldson for preparing the histological material, Mrs Vivienne Harris for technical assistance and Mr Robert King for providing visually inexperienced lambs.

V. S. RAMACHANDRAN*
P. G. H. CLARKE
D. WHITTERIDGE

University Laboratory of Physiology,
Parks Road,
Oxford, UK

Received 25 April; accepted 9 June 1977.

*Present address: The Psychological Laboratory, University of Cambridge, Cambridge, UK.

¹ Hering, E. in *Handbuch der Physiologie* 3 (1) (ed. Hermann, L.): see English translation by C. A. Radde, *Spatial Sense and Movements of the Eyes* (American Academy of Optometry, Baltimore, 1942).

² Helmholtz, H. von in *Treatise on Physiological Optics*, 3, (ed. Southall, J. P. C.) ch. 33 (Dover, New York, 1962).

³ Barlow, H. B., Blakemore, C. & Pettigrew, J. D. *J. Physiol., Lond.* **193**, 327-342 (1967).

⁴ Nikara, T., Bishop, P. O. & Pettigrew, J. D. *Expl Brain Res.* **6**, 353-372 (1968).

⁵ Hubel, D. H. & Wiesel, T. N. *Nature* **225**, 41-42 (1970).

⁶ Clarke, P. G. H., Donaldson, I. M. L. & Whitteridge, D. *J. Physiol., Lond.* **256**, 509-526 (1976).

⁷ Wiesel, T. N. & Hubel, D. H. *J. Neurophysiol.* **28**, 1060-1072 (1965).

⁸ Blakemore, C. & Cooper, G. F. *Nature* **228**, 477-478 (1970).

⁹ Hirsch, H. V. B. & Spinelli, D. N. *Science* **168**, 869-871 (1970).

¹⁰ Barlow, H. B. *Nature* **258**, 199-204 (1975).

¹¹ Pettigrew, J. D. *J. Physiol., Lond.* **237**, 49-74 (1974).

¹² Keating, M. J. *Br. Med. Bull.* **30**, 145-151 (1974).

¹³ Pettigrew, J. D. *Nature* **264**, 753-754 (1976).

¹⁴ Grobstein, P. & Chow, K. L. *Science* **190**, 352-358 (1975).

¹⁵ Blakemore, C. & Van Sluyters, R. C. *J. Physiol., Lond.* **248**, 663-716 (1975).

¹⁶ Shlaer, R. *Science* **173**, 638-641 (1971).

¹⁷ Gibson, E. J. & Walk, R. D. *Sci. Am.* **202**, 64-71 (1960).

¹⁸ Walk, R. D. & Gibson, E. J. *Psychol. Monogr.* **75**, 1-44 (1961).

¹⁹ V. Geist Mountain Sheep, 246 (University of Chicago Press, Chicago, 1971).

²⁰ Merrill, E. G. & Ainsworth, A. *Med. Biol. Engng* **10**, 662-672 (1972).

²¹ Movshon, J. A. *J. Physiol., Lond.* **261**, 125-174 (1976).

²² Wiesel, T. N. & Hubel, D. H. *J. comp. Neurol.* **158**, 307-318 (1974).

Early hyperthyroidism alters the distribution of mossy fibres in the rat hippocampus

DAILY injections of thyroid hormone initiated on the day of birth and continued for several weeks, significantly accelerate the differentiation of cerebellar granule cells¹⁻³, which are formed after birth⁴. Since most of the hippocampal granule cells are also generated postnatally, early hyperthyroidism was chosen as a means of studying possible mechanisms involved in the formation of synapses between granule cell axons (mossy fibres) and pyramidal cell dendrites. This note provides the light microscopic demonstration, with the Timm silver sulphide method⁵, that hyperthyroidism in the rat consistently results in the formation of ectopic hippocampal mossy fibres. Electron microscopic and Golgi studies are in progress and will be published separately.

Basic mechanisms governing the formation of neuronal circuitries are as yet poorly understood. The spatial segregation of afferents along defined regions of the postsynaptic surface in neurones such as the cerebellar Purkinje cells and hippocampal pyramidal and granule cells indicate a high degree of specificity during neural ontogeny. At present, it is unclear how intrinsic neuronal properties, temporal factors, specific affinities and recognition processes interact in such development. The strict segregation of afferents along the pyramidal cell surface, in the hippocampus, first clearly conceived by Cajal⁶ and further explored by Lorente de No⁷, as well as the unique synaptic relationship between mossy fibres and the large protuberances (or 'excrescences') on the apical dendritic shaft of the pyramidal cell, make this an excellent region to study synaptogenesis, especially the relative parts played by the pre- and postsynaptic elements⁸. Genetically associated variations of this system have recently been reported in the mouse⁹.

Since the synaptic boutons of the mossy fibres contain a metal (most likely zinc)¹⁰, which can be stained histochemically using the Timm silver sulphide procedure⁵, an easy and specific bulk method is available for light microscopic screening of large numbers of sections with respect to alterations in the distribution of granule cell axons. Hooded rats (males, 8 per litter) were made hyperthyroid by the subcutaneous injection of daily doses of 5-15 µg L-thyroxine in 0.1 ml physiological saline from the day of birth until day 15. Control animals were injected daily with 0.1 ml physiological saline during the same period. All animals were killed 30 d postnatally, by cardiac perfusion with 1.19% sodium sulphide and 5% polyvinyl pyrrolidone (R/P) in 1 l of 60% ethanol, preceded by a short prewash with physiological saline under choral hydrate anaesthesia. Brains were dehydrated, embedded in Paraplast, and sectioned serially along the horizontal plane. Slides were stained using Timm the silver sulphide method for the histochemical visualisation of mossy fibres as described extensively by others^{5,9,11}. A total of eight hyperthyroid and eight control animals were used in this study, but more than 20 hyperthyroid animals have been examined, when rats injected with thyroxine according to different schedules are included.

In controls, as in the normal albino rat, the mossy fibres pass into the hilus of the fascia dentata, then course along the pyramidal cell layer largely transverse to the septotemporal axis of the hippocampal formation^{7,12,13}. Most of these axons synapse *en passant* with the excrescences on the apical dendrites of pyramidal cells. These fibres form the suprapyramidal

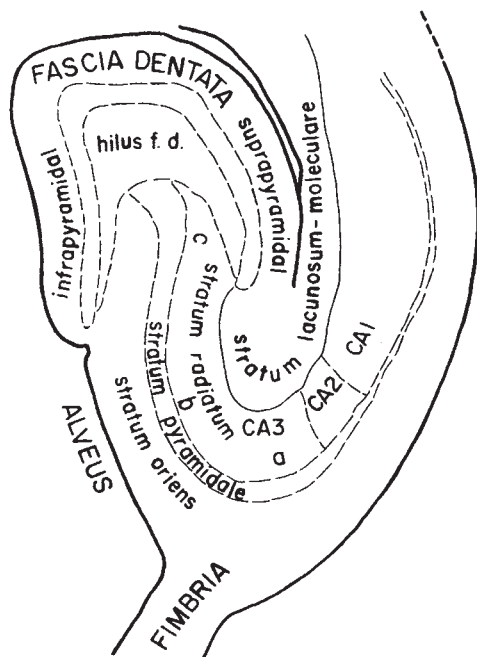


Fig. 1 Diagram of Ammon's horn in the horizontal plane to indicate nomenclature. Regio inferior comprises CA3 and CA2; Regio superior, CA1. Although it is unclear whether CA2 exists as a defined area and to what extent its pyramidal cells receive mossy fibre terminals^{13,15}, we have designated with this term the border zone between Regio inferior and Regio superior within which the supradiamidal mossy fibre bundle terminates, in accordance with the terminology used by Chronister and White¹⁵, Angevine¹⁹ and Hine and Das²¹.

mossy fibre bundle, extending as far as the border between Regio inferior (CA3–CA2) and Regio superior (CA1) (see terminology Fig. 1). Others take a more ventral, intra- and infrapiamidal route for a short distance, synapsing with excrescences of both the basal and apical dendrites of pyramidal cells in subfield CA3_b before joining the supradiamidal bundle (Fig. 2a)^{13–15}.

At most ventral and dorsal levels (not illustrated) some infrapiamidal mossy fibre terminals are also present in CA2 and CA3_{a-b}. It is known, however, that at the middle septotemporal level, as defined by Haug¹¹, the infrapiamidal zone in CA2 and CA3_{a-b} contains few, if any, mossy fibre terminals (Fig. 2a). It is at this level of the hippocampus that we found the

most clear-cut changes in the experimental animals. As shown in Fig. 2b, at this level in the hyperthyroid rat a dense layer of Timm reaction product, whose thickness is approximately one-third of the supradiamidal mossy fibre layer, is consistently located in an infrapiamidal position in CA3_{a-b}, and CA2, reaching the border with CA1. Rows of Timm granules cross the pyramidal cell layer, suggesting that many infrapiamidal mossy fibres originate from the supradiamidal bundle. A number of Golgi rapid sections available now support the former view, although it is also possible that some fibres originate from the infrapiamidal bundle. Also, preliminary electron microscopic studies show bundles of infrapiamidal mossy fibres in hyperthyroid rats with *en passant* mossy swellings synapsing on excrescences of the basal dendritic stems of pyramidal cells in CA3_{a-b}. In normal animals the basal dendrites of pyramidal cells receive little, if any, mossy fibre input in this field of Ammon's Horn, and have smooth shafts with few and simple spinous evaginations.

Why the lower border of the mossy fibre bundle is drastically changed and the transverse (between Regio inferior and Regio superior) and upper (towards the stratum radiatum) borders are not, may depend on the time at which the administered hormone starts to interfere with the morphogenetic process.

Various studies on hippocampal development^{16–23} indicate that in addition to specific chemical affinities and recognition phenomena, temporal factors are of considerable morphogenetic importance. Complex spatio-temporal relationships between the time of origin and subsequent differentiation of granule cells and pyramidal cells have been demonstrated. These patterns include the start of granule cell differentiation in the supradiamidal (lateral) blade of the dentate gyrus earlier than in the infrapiamidal (medial) blade and the birth of granule cells nearest the molecular layer before those towards the hilus^{16,17} an 'inside-out' pattern of pyramidal cell generation^{19,21} in that the deep pyramidal cells are born before those more superficial in the pyramidal layer, and the formation of apical pyramidal cell dendrites earlier than basal dendrites²². Such spatio-temporal relationships between the differentiation of hippocampal granule cells and their pyramidal cell targets could provide an ideal system for the formation of discrete mossy fibre pyramidal cell connections, as suggested by recent studies in the mutant mouse²³.

How the position of individual mossy fibres within the supradiamidal bundle is determined remains to be ascertained. In the cerebellum the parallel fibre growth is appositional, that is, the lower fibres are formed first and the most superficial last^{24,25}. The existence of a chronological-positional order in

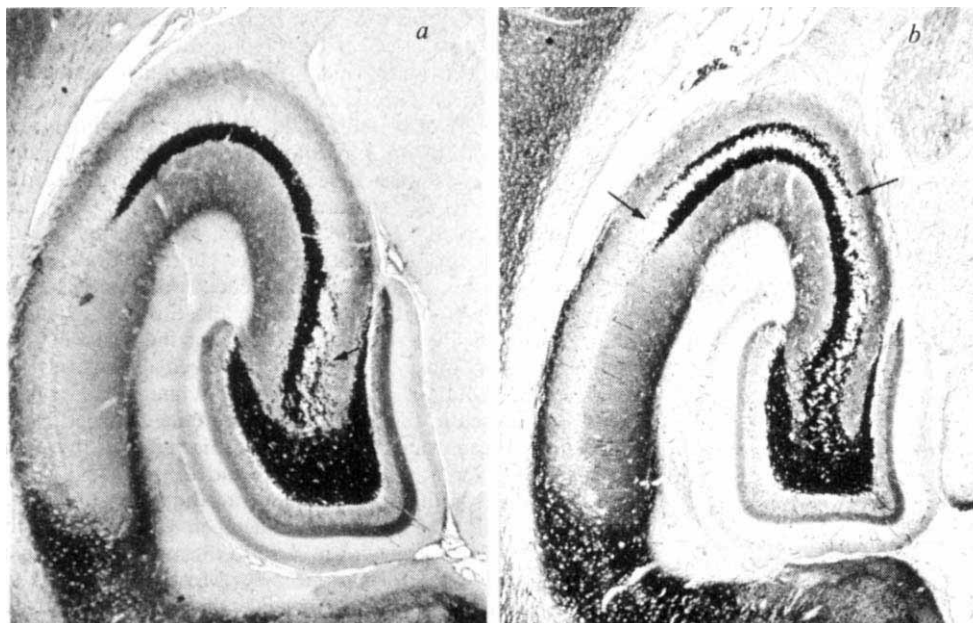


Fig. 2a, Horizontal hippocampal section from saline-injected 30-d-old rat; Timm staining method; middle septotemporal level of the hippocampus as defined by Haug¹¹ (his Fig. 2c). The Timm precipitate marks the hilus fascia dentatae, the mossy fibre layer in CA3_{abs} and that part of CA3_e where mossy fibre bundles run through the pyramidal layer reaching an infrapiamidal position (arrow). In CA2 and especially CA3_{a-b} the infrapiamidal zone shows only scattered Timm-positive granules (unlabelled). $\times 30.2$
b, Horizontal hippocampal section from a 30-d-old rat injected daily with 10 μ g L-thyroxine from birth to day 15. Middle septotemporal level of the hippocampus as defined by Haug¹¹. Note the conspicuous Timm staining in the infrapiamidal zone of CA2 and CA3_{a-b} (arrows). $\times 30.2$

the hippocampal mossy fibre bundle is suggested by the fact that with postnatal hyperthyroidism the ectopic synapses are formed infra- and not suprapyramidally.

Other available data suggest that the abnormally expanded mossy fibre area in the hyperthyroid rat may be a consequence of an exaggerated growth of postnatally-generated granule cell axons and of an acceleration of granule cell differentiation. An effect of thyroid hormone on axonal growth has been indicated in recent developmental and regeneration studies^{1,26}. Moreover, in the cerebellum, early hyperthyroidism accelerates the peak of postnatal granule cell differentiation². This experimental condition also results in a permanent deficit in total cell number in the cerebrum and cerebellum²⁷, apparently caused mainly by early cessation of proliferation of germinal cells which results in a smaller number of postnatally generated microneurons. It would be interesting to know whether the number of hippocampal granule cells is also reduced. The effect of thyroid hormone on growth of the hippocampal mossy fibres could be more powerful than it seems from our work if the expanded mossy fibre area were produced by a smaller number of granule cells.

Although a primary effect on the presynaptic element seems probable, at present neither a direct influence of thyroid hormone on the pyramidal neurones, nor an indirect one by way of other hormones can be ruled out, especially since the dendritic tree of the pyramidal neurones is still undergoing development during the hormonal treatment. The aberrant mossy fibre terminals may occupy vacant postsynaptic sites, successfully compete for existent synaptic loci, or induce the formation of new ones. These questions and whether the ectopic mossy fibres are functionally active, remain to be determined.

Our results are somewhat germane to the demonstration of axonal 'sprouting' and 'expansion' of undamaged terminal areas after partial de-afferentation of various cortical and subcortical areas during development²⁸. The hormonal treatment, however, affords the possibility of producing heterotopic synapses without the degeneration of neuronal elements and the glial cell proliferation which follows de-afferentation. The expanded target area of the mossy fibres in the hyperthyroid rat, represented by the accessory infrapyramidal bundle in CA2 and CA3_{a-b}, indicates that the normal pattern of axonal growth can be altered experimentally at a site where segregation of afferents appeared so strict as to be paradigmatic.

This work was supported by NIH grant 09904-06.

JEAN M. LAUDER
ENRICO MUGNAINI

Laboratory of Neuromorphology,
Department of Biobehavioral Sciences,
The University of Connecticut,
Storrs, Connecticut 06268

Received 15 March; accepted 17 May 1977.

- ¹ Lauder, J. M., in *Thyroid Hormones and Brain Development* (ed. Grave, G. D.) (Raven, New York, 1977).
- ² Nicholson, J. L. & Altman, J. *Brain Res.* **44**, 13-23 (1972).
- ³ Nicholson, J. L. & Altman, J. *Brain Res.* **44**, 25-36 (1972).
- ⁴ Altman, J. *J. comp. Neurol.* **136**, 269-294 (1969).
- ⁵ Timm, F. *Deutsch. Z. ges. gerichtl. Med.* **46**, 706-711 (1958).
- ⁶ Ramón y Cajal, S. *The Structure of Ammon's Horn* (transl. by Kraft, L. M.) (C. C. Thomas, Springfield, 1968).
- ⁷ Lorente de Nó, R. *J. Psychol. u. Neurol.* **46**, 113-177 (1934).
- ⁸ Mugnaini, E., in *Excitatory Synaptic Mechanisms* (eds Andersen, P. & Jansen, J. K. S.), 149-169 (Universitetsforlaget, Oslo, 1970).
- ⁹ Barber, R. P., Vaughn, J. E., Wimer, R. E. & Wimer, C. C. *J. comp. Neurol.* **156**, 417-434 (1974).
- ¹⁰ von Euler, C. in *Physiologie de L'hippocampe* (ed. Passouant, P.), 135-145 (Quai Anatole-France, Paris, 1962).
- ¹¹ Haug, F.-M., S. Z. *Anat. Entwickl.-Gesch.* **145**, 1-27 (1974).
- ¹² Blackstad, T. W. & Kjærheim, Å. *J. comp. Neurol.* **117**, 133-159 (1961).
- ¹³ Blackstad, T. W., Brink, K., Hem, J. & Jeune, B. *J. comp. Neurol.* **138**, 433-450 (1970).
- ¹⁴ Hamlyn, L. H. *J. Anat.* **96**, 112-120 (1962).
- ¹⁵ Chronister, R. B. & White, L. E. Jun. in *The Hippocampus*, 1 (ed. Isaacson, R. L. & Pribram, K. H.), 9-39 (Plenum, New York, 1975).
- ¹⁶ Bayer, S. A. & Altman, J. *J. comp. Neurol.* **158**, 55-80 (1974).
- ¹⁷ Schlessinger, A. R., Cowan, W. M. & Gottlieb, D. I. *J. comp. Neurol.* **159**, 149-176 (1975).
- ¹⁸ Schwartz, I. R., Pappas, G. D. & Purpura, D. P. *Expl Neurol.* **22**, 394-407 (1968).
- ¹⁹ Angevine, J. B., Jun. *Expl Neurol. Suppl.* **2**, 1-70 (1965).
- ²⁰ Gottlieb, D. I. & Cowan, W. M. *Brain Res.* **41**, 452-456 (1972).
- ²¹ Hine, R. J. & Das, G. D. Z. *Anat. Entwickl.-Gesch.* **144**, 173-186 (1974).
- ²² Purpura, D. P. & Pappas, G. D. *Expl Neurol.* **22**, 379-393 (1968).
- ²³ Vaughn, J. E., Matthews, D. A., Barber, R. P., Wimer, C. C. & Wimer, R. E. *J. comp. Neurol.* **173**, 41-52 (1977).
- ²⁴ Altman, J. *J. comp. Neurol.* **145**, 353-398 (1972).
- ²⁵ Rakic, P. *J. comp. Neurol.* **146**, 335-354 (1972).
- ²⁶ Fertig, A., Kiernan, J. A. & Seyan, S. S. A. S. *Expl Neurol.* **33**, 372-385 (1971).
- ²⁷ Balázs, R., Kovacs, S., Cocks, W. A., Johnson, A. L. & Eayrs, J. T. *Brain Res.* **25**, 555-570 (1971).
- ²⁸ Lynch, G., Stanfield, B. & Cotman, C. W. *Brain Res.* **59**, 155-168 (1973).

Generality of oestrogen stimulation of peroxidase activity in growth responsive tissues

OVER 20 years ago, Stotz *et al.*¹ reported that administration of oestrogen to rats effected an increase in the peroxidase (EC 1. 11. 1. 7) activity of the uterus. Subsequently, this stimulation of uterine peroxidase activity was attributed to an influx of eosinophils into the tissue². More recent evidence, both biochemical³⁻⁵ and histochemical⁶⁻⁷, clearly indicates a uterine origin for the enzyme. On the basis of histochemical peroxidase assays, we proposed that peroxidase may be a useful marker protein response elicited by oestrogen in those rat tissues, like uterus⁸, vagina⁸ and mammary tumour⁹, whose growth is regulated by oestrogen. Recent data¹⁰ on the dose and steroid dependence of rat uterine peroxidase induction also supports this proposition. To be a meaningful marker for the effect of oestrogen on growth responsive tissues, the peroxidase induction should be demonstrable in animals other than the rat and should show a reasonable tissue specificity. This communication reports on the generality and specificity of oestrogen regulation of peroxidase activity.

Rats (Sprague-Dawley strain), mice (Swiss Albino strain), and hamsters (Golden Syrian strain) were obtained from ARS/Sprague-Dawley, Madison, Wisconsin. Guinea pigs (Hartley strain) were obtained from Camm Research, Wayne, NJ. Animals were injected with oestradiol-17 β , (Sigma) 40 μ g per kg body weight in 25% ethanol-saline or vehicle alone, 44 and 20 h before decapitation. Trimmed tissues were homogenised in cold 10 mM Tris-HCl, pH 7.2 buffer, 25-50 mg tissue per ml, centrifuged 45 min at 39,000g 2 °C and the peroxidase activity solubilised by rehomogenising the sediments with 0.5 M CaCl₂ in 10 mM Tris buffer, pH 7.2. The extracts were collected after 45 min, 39,000g centrifugation at 2 °C. Although both the hypotonic and CaCl₂ extracts were assayed for peroxidase activity, only minimal activity was found in the original 10 mM Tris supernatants (<5% of total).

Peroxidase assays were performed by modification of the method of Himmelhoch *et al.*¹¹, at 25 °C. The assay mixture, 3.0 ml total volume, contained 13 mM guaiacol and 0.3 mM H₂O₂ in 10 mM Tris buffer, pH 7.2 containing 0.5 M CaCl₂. The reaction was started by the addition of enzyme (extract) and initial rates of guaiacol oxidation were determined from the increase in absorbance at 470 nm for the first minute. An enzyme unit was defined as the amount of enzyme required to produce an increase of one absorbance unit per minute in these assay conditions.

Table 1 shows the effect of two injections of oestradiol on the uterine wet weight and uterine peroxidase activity in four species of laboratory animals. In each case, there was a significant increase in both uterine weight and uterine peroxidase content but it is apparent that there were appreciable differences in the uterine sensitivity of these various animals to oestrogen, given at identical doses based on body weight. However, the relative increases in peroxidase content closely parallel the relative increases in weight of the uteri. Thus, the hamster showed the smallest increase in uterine weight (35%) and the smallest

Table 1 Oestradiol stimulation of the uterus of various species

Animal (age/wt)	Group*	Uterine wt (mg)	Uterine peroxidase activity Units (per g $\pm$ s.e.m.)	mUnits per uterus
Hamster (25 d/46 g)	C	45.8	0.064 $\pm$ 0.018	2.9
	E	62.4	0.132 $\pm$ 0.016	8.2
	(E/C)	(1.36)	(2.06)	(2.9)
Guinea pig (23 d/210 g)	C	232	0.18 $\pm$ 0.04	42
	E	534	0.83 $\pm$ 0.17	443
	(E/C)	(2.30)	(4.61)	(10.5)
Rat (30 d/54 g)	C	25.2	0.27 $\pm$ 0.09	6.8
	E	84.3	24.6 $\pm$ 7.3	2074
	(E/C)	(3.35)	(91.1)	(305)
Mouse (28 d/19 g)	C	7.8	0.1 $\pm$ 0.04	0.78
	E	35.0	29.3 $\pm$ 10	1,026
	(E/C)	(4.49)	(293)	(1,315)

*Animals (4–10 per group) received subcutaneous injections of vehicle(C) or oestradiol (E) 40 μ g kg⁻¹ body weight, 44 and 20 h before killing. E/C is ratio of weight or peroxidase of oestrogen treated to control uteri.

increase in uterine peroxidase (2-fold). The most dramatic uterine sensitivity to oestradiol was seen in the mouse in which the uterus grew about 4.5-fold and showed almost a 300-fold increase in peroxidase concentration. The rat and mouse showed remarkable (300- and 1,300-fold) increases of total uterine content of peroxidase in 44 h of oestradiol treatment.

The oestrogen stimulation of peroxidase activity seems to be restricted to growth-responsive tissues (Table 2). Significant increases in peroxidase concentration are seen in both the uterus and vagina of the immature rat given oestradiol, but no peroxidase is found in the pituitary. Although small to large concentrations of peroxidase can be found in the kidney, thyroid, lung and spleen, no appreciable change in the peroxidase content of these tissues occurs on oestradiol treatment. Other tissues assayed, specifically ovary, adrenal and liver, had no significant peroxidase in the oestrogen-treated or untreated rat. Although the peroxidase content of the thyroid of the immature animal is low, the adult rat thyroid peroxidase content (about 40 units g⁻¹) was not influenced by oestrogen treatment.

Figure 1 shows the peroxidase content of some human oestrogen-responsive tissues. The endometrium and myometrium were obtained by physical separation from small samples of human uterus. While care was taken to obtain pure endometrium, it is probable that the myometrium contained some residual glandular tissue. Clearly, the peroxidase content of uterine endometrium exceeds that of the myometrium. Samples of human breast cancer tissue showed a wide range of peroxidase content. Nine of the 39 samples (23%) had no peroxidase, while 11 (28%) had an appreciable amount, that is greater than 1 unit g⁻¹.

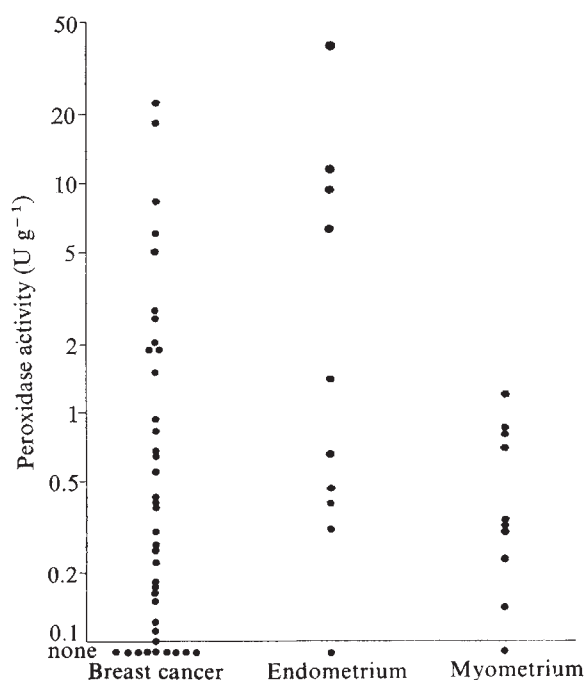
Up to the present, there has not seemed to be any

particularly meaningful protein marker for oestrogen action in the mammalian uterus. Although the induced protein or IP, first described by Gorski *et al.*¹² has generated considerable interest in this regard, a recent report¹³ suggests that this protein may be limited to the rat uterus; it was not found in the uterus of the mouse, rabbit, hamster or guinea pig, or in the rat vagina. Whereas certain enzymes, especially those involved with carbohydrate metabolism, have been found to increase in the uterus of the oestrogen treated animal, the extent of these increases are generally small, at best 5–10-fold¹⁴, and largely reflect the increased metabolic activity of the tissue.

Uterine peroxidase activity seems to be a good marker for oestrogen stimulation of growth. As shown in this report, oestrogen effects a growth-related increase in peroxidase concentration in the uterus of various species. Furthermore, the oestradiol stimulation of the uterine peroxidase content of the immature mouse and rat uterus, as much as a 1,000-fold increase, is certainly one of the most dramatic effects of oestrogen. Peroxidase stimulation by oestrogen is limited to the growth-responsive normal tissues, uterus and vagina, and is not seen in the pituitary where the response to oestrogen is largely functional. Oestrogen-independent tissues of the rat show no significant stimulation of peroxidase content, whether or not the tissue of the untreated animal contained any enzyme.

An earlier report¹⁵ indicated that the peroxidase content of human endometrial brushings showed a variation coinciding with the menstrual cycle. Since the human uterine samples reported here were from pre-menopausal patients, the range of peroxidase content probably reflects menstrual cycle variation. The high endometrial peroxidase concentrations agree quantitatively with the concentrations found in the oestrogen-stimulated rodent uterus (Fig. 1 and Table 1), suggesting that studies on the regulation of peroxidase in the rodent uterus are quite relevant to the human.

It is particularly interesting to find that a significant

Fig. 1 Peroxidase content of human uterus and breast cancer. Calcium chloride extracts of samples of human endometrium, myometrium and breast cancer tissue assayed for peroxidase content.**Table 2** Tissue specificity of oestrogen effect on peroxidase activity

Rat tissue	Peroxidase activity (units per g)	
	Control	E2 treated*
Uterus	0.16	32.4
Vagina	0.0	5.8
Pituitary	0.0	0.0
Kidney	0.4	0.5
Thyroid	0.4	0.5
Lung	15.6	20.4
Spleen	186	188

*Immature female rats given oestradiol-17 β , 40 μ g per kg body wt, subcutaneously 44 and 20 h before killing.

number of human breast cancer specimens contain appreciable amounts of peroxidase activity. An earlier report from our laboratory described the oestrogen regulation of peroxidase in the carcinogen-induced mammary tumours of the rat⁹. Data of the last 5 yr have indicated that essentially only those patients whose breast cancer tissue contains appreciable amounts of oestrogen receptor respond to endocrine therapies^{16,17}. Since, however, at least a third of the patients with oestrogen receptor positive tumours do not respond, it is apparent that the presence of oestrogen receptor is an essential, but not necessarily sufficient, condition for response. It is possible that use of an assay of a biochemical parameter, possibly peroxidase, which reflects tissue hormone-dependence may enhance the accuracy of prediction of response in those breast cancer patients whose tumours contain oestrogen receptor. Such studies are in progress.

The generality and specificity of the oestrogen stimulation of peroxidase activity in tissues which are stimulated to grow by oestrogen recommends this marker as a meaningful parameter for the more detailed studies of steroid hormone regulation of gene function as related to growth. Further study of peroxidase content of normal and neoplastic human tissues can provide a better understanding of hormonal regulation of these tissues and possibly a more accurate diagnosis of oestrogen-dependent neoplasms.

This work was supported by a contract (NO1 CB-23873) from the National Cancer Institute, USPHS. The technical assistance of R. Garay and R. Dizon is gratefully acknowledged.

C. RICHARD LYTTLE
EUGENE R. DESOMBRE

Ben May Laboratory for Cancer Research,
The University of Chicago,
Chicago, Illinois 60637

Received 3 May; accepted 6 June 1977

- ¹ Lucas, F. V., Neufeld, H. A., Utterback, J. G., Martin, A. P. & Stotz, E. J. *biol. Chem.* **214**, 775-780 (1955).
- ² Klebanoff, S. J. *Endocrinology* **76**, 301-311 (1965).
- ³ Jellinck, P. H. & Lyttle, C. R. *Adv. Enzyme Reg.* **11**, 17-32 (1972).
- ⁴ Jellinck, P. H., McNabb, T., Cleveland, S. & Lyttle, C. R. *Adv. Enzyme Reg.* **14**, 447-465 (1976).
- ⁵ Lyttle, C. R. & Jellinck, P. H. *Biochem. J.* **160**, 237-241 (1976).
- ⁶ Brokelmann, J. & Fawcett, D. W. *Biol. Reprod.* **1**, 59-71 (1969).
- ⁷ Churg, A. & Anderson, W. A. *J. Cell Biol.* **62**, 449-459 (1974).
- ⁸ Anderson, W. A., Kang, Y.-H. & DeSombre, E. R. *J. Cell Biol.* **64**, 668-681 (1975).
- ⁹ DeSombre, E. R., Anderson, W. A. & Kang, Y.-H. *Cancer Res.* **35**, 172-179 (1975).
- ¹⁰ Lyttle, C. R. & DeSombre, E. R. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ¹¹ Himmelhoch, S. R., Evans, W. H., Mage, M. G. & Peterson, E. A. *Biochemistry* **8**, 914-921 (1969).
- ¹² Notides, A., Gorski, J. *Proc. natn. Acad. Sci. U.S.A.* **56**, 230-235 (1966).
- ¹³ Kram, R., Vokaer, A., Bompiani, A. & Iacobelli, S. J. *Steroid Biochem.* **5**, 342-343 (1974).
- ¹⁴ Baquer, N. Z. & McLean, P. *Biochem. biophys. Res. Commun.* **48**, 729-734 (1972).
- ¹⁵ Lucas, F. V., Carnes, V. M., Schmidt, H. J., Sipes, D. R. & Hall, D. G. *Am. J. Obstet. Gynec.* **88**, 965-967 (1964).
- ¹⁶ McGuire, W. L., Carbone, P. P. & Vollmer, E. P. (eds) *Estrogen Receptors in Human Breast Cancer* (Raven, New York, 1975).
- ¹⁷ Jensen, E. V. & DeSombre, E. R. *Adv. clin. Chem.* **19**, 57-89 (1976).

active against Chagas' disease is time-consuming, costly and hazardous². Because of the danger of infection when working with *T. cruzi*, it has been suggested by Baker³ that other species of the subgenus *Schizotrypanum* of the genus *Trypanosoma* could be used as models for studying this parasite. *T. dionisii*, isolated from the bat, *Pipistrellus pipistrellus*, by Baker and Thompson⁴, seems to be a particularly suitable choice because of its apparent non-pathogenicity in man³. The experiments described here show that *T. dionisii* can replace *T. cruzi* in *in vitro* drug screens.

T. dionisii (from J. R. Baker, Molteno Institute, University of Cambridge) and the Sonya strain of *T. cruzi*⁵ were maintained in modified LIT medium⁶ at 28 °C. This was supplemented with 10% (by volume) heat-inactivated new-born calf serum for *T. cruzi* and with the same concentration of heat-inactivated rabbit serum for *T. dionisii*. Four-day-old cultures of each organism were used to inoculate fresh medium containing drugs to an initial cell density of 10⁶ organisms per ml. The effects of the drugs on growth were observed over a 72 h incubation period. The drugs used included representatives of each of the eight chemical structures so far tested clinically against *T. cruzi*¹. The results (Table 1) showed that the drug sensitivities of the

Table 1 Effects of drugs known to be active in animal screens on the culture epimastigotes of *T. dionisii* and *T. cruzi*

Drug* (µg ml ⁻¹)	Growth (% of control growth)			
	<i>T. dionisii</i> 24 h	<i>T. dionisii</i> 72 h	<i>T. cruzi</i> 24 h	<i>T. cruzi</i> 72 h
Bayer 7602 Ac (bisquinaldine)	100	98	100	102
Spirotrypan (arsenobenzene)	1	113	120	98
	10	12	18	2
	100	0	0	0
Ethidium Br (phenanthridine)	1	58	41	97
	10	59	10	56
	100	26	5	15†
Pentaquine (8-aminoquinoline) diphosphate	1	93	91	27
	10	46	5	25
	100	76†	0	7
Cordycepin (purine analogue)	10	76	60	ND‡
	100	30	4	ND‡
Lampit (5-nitrofurantoin)	1	106	91	90
	10	6	0	97
	100	0	0	0
SQ 18506 (5-nitrofurantoin)	1	0	0	0
Metronidazole (5-nitroimidazole)	10	102	98	ND‡
	100	96	47	ND‡
Ro 7-1051 (2-nitroimidazole)	1	66	70	95
	10	48	9	89
	100	7	0	4

*Detailed chemical structures in ref. 1.

†Abnormal appearance.

‡Not determined.

Comparative drug sensitivities of culture forms of *Trypanosoma cruzi* and *Trypanosoma dionisii*

At least 12 million people suffer from Chagas' disease in South America, but despite intensive efforts over the last 65 yr, no treatment effective against all stages of the disease has emerged¹. *Trypanosoma cruzi* the causative agent of the disease, develops intracellularly in the tissues of the heart and alimentary tract. Such development, together with the absence of effective chemotherapy, means that drug testing for agents

two species are almost identical. This suggests that *T. dionisii* can be used to replace *T. cruzi* in drug screens of this kind. Primary *in vitro* drug screens, however, are never entirely satisfactory since some false-positive and, more significantly, a few false-negative results will appear⁷. The absence of activity with Bayer 7602 Ac, which is quite active in animal screens and the detection of activity with cordycepin, which is relatively inactive *in vivo*, indicates that *T. dionisii in vitro* is not an exception to these limitations. But, if the alternative is no routine testing at all, as seems to be the situation now¹, we suggest that the use of *T. dionisii in vitro* as a safe, quick and

inexpensive primary screen for detecting activity against Chagas' disease should be considered.

This work was supported by the Ministry of Overseas Development, London and the WHO, Geneva.

M. GABORAK
J. L. DARLING
W. E. GUTTERIDGE

Biological Laboratory,
University of Kent,
Canterbury, UK

Received 13 May; accepted 3 June 1977.

¹ Gutteridge, W. E. *Trop. Dis. Bull.* 73, 699-705 (1976).

² Gutteridge, W. E. *P.A.H.O. Sci. Publ.* No. 318, 255-262 (1976).

³ Baker, J. R. *Trans. R. Soc. trop. Med. Hyg.* 70, 126-127 (1976).

⁴ Baker, J. R. & Thompson, G. B. *Trans. R. Soc. trop. Med. Hyg.* 65, 427 (1971).

⁵ Garnham, P. C. C. *Trans. R. Soc. trop. Med. Hyg.* 50, 613 (1956).

⁶ Gutteridge, W. E., Knowler, J. & Coombes, J. D. *J. Protozool.* 16, 521-525 (1969).

⁷ Ryley, J. F. & Wilson, R. G. *Parasitology* 73, 137-148 (1976).

Eosinophils and not lymphoid K cells kill *Trypanosoma cruzi* epimastigotes

CHAGAS' disease caused by the protozoan *Trypanosoma cruzi* remains a medical problem of immense proportions in Latin America, and yet the study of the pathogenesis and immunology of the disease can reasonably be said to be in its infancy. The

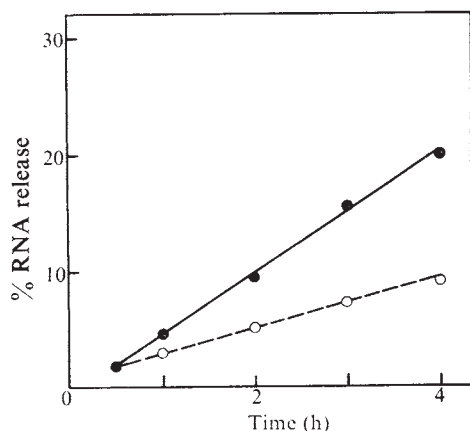


Fig. 1 Showing the time course of cytotoxicity of normal rat spleen cells in the presence (●) and absence (○) of antibody. An inbred line of white rats (O strain) was used. The antiserum was prepared by injecting rats with 10^6 blood forms from parasitaemic mice, and bleeding at 14 d. Culture forms of *T. cruzi* (Y strain) were inoculated into 1 ml of Warren's medium⁹ containing $10 \mu\text{Ci}$ of ^3H -uridine (43 Ci mmol^{-1}). After 5 d, the parasites were washed four times with medium (RPMI:1640 containing 10 mM HEPES and 5% inactivated foetal calf serum), resuspended to a concentration of 10^6 ml^{-1} and distributed (100 μl) in small plastic tubes. Spleen cells were added in a volume of 100 μl to give a ratio of 20:1 (cells:parasites). The antiserum diluted 1:100 was added as a further 100 μl (total volume 300 μl). The tubes were incubated at 37°C , and at the times indicated, tubes were removed from the incubator, resuspended and centrifuged. After centrifugation, 200 μl of the supernatant was carefully removed to a counting vial. Total isotope in 10^5 parasites was determined by placing 100 μl of the original parasite suspension in a counting vial. The parasites were lysed by adding 100 μl of 1% Triton X-100. A 2-ml aliquot of a water-miscible scintillation fluid (1 vol. Triton X-100 and 2 vol. toluene-based scintillation fluid) was then added to each vial. The vials were counted in a liquid scintillation counter, and after correcting for background, % RNA release was calculated: $1.5C \times 100/T$ (T = the total counts in 10^5 parasites (mean of 4 replicates) and C = counts in each test vial (2/3 of supernatant counts)). The results were analysed by analysis of variance using logarithmic transformation, and Duncan's multiple range test¹⁰. All points shown are significantly different at the 5% level or less. Control release in the presence of antibody but absence of cells was not significantly different from (○), and has not been plotted.

demonstration of the protective role of humoral immunity *in vivo*¹ and of antibody-dependent cell-mediated cytotoxicity against *T. dionisii in vitro*² has raised the question that this phenomenon may be important biologically in Chagas' disease and might be mediated by K cells normally assayed as killer cells against antibody-coated tumour cells³. Here we address the second part of the question, and show that K cells in rat spleen have no detectable activity against *T. cruzi*, but that the activity resides in cell preparations rich in eosinophils.

It has been necessary to develop a simple and economical isotopic technique to assay parasite death. It has been noted previously (and we have confirmed it) that trypanosomes do not incorporate sufficient ^{61}Cr to provide a cytotoxic assay². Those authors described a technique using $^{99\text{m}}\text{Tc}$, but the limited availability and short half life (6 h) of this isotope provide considerable practical difficulties. Based on experiments with tumour cells where the release of RNA was found to provide a sensitive assay for cytotoxicity⁴, we investigated the use of ^3H -uridine to label the RNA in the parasite. We have found (to be published) that, as is the case with tumour cells, RNA is released more rapidly than DNA and more completely than protein from killed parasites. Culture forms (largely epimastigotes) were labelled by growing them in a medium containing ^3H -uridine and >95% of the incorporated label was precipitable by 5% trichloroacetic acid (and thus RNA). Figure 1 shows the release of RNA from *T. cruzi* by normal rat spleen cells in the presence and absence of specific antibody to the parasite, in medium containing inactivated foetal calf serum.

To investigate the properties of the cells mediating this cytotoxic reaction, the spleen cells were fractionated by Ficoll-Hypaque into high and low density fractions⁵, followed by nylon wool columns to separate the cells on the basis of their adherence properties⁶. These fractions were tested against antibody-coated P815 cells as an assay for K cells, and against antibody-coated *T. cruzi*. It has already been shown that K cells are low density, non-adherent cells^{4,7}.

The activity against *T. cruzi* (Fig. 2) is found in the high density fraction (P) which contains very little activity against the

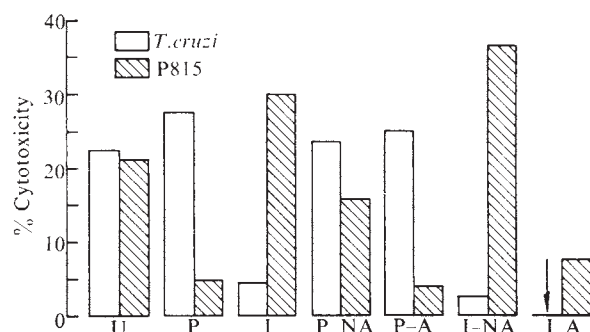


Fig. 2 Comparison of cell fractions against P815 mastocytoma cells using a ^{51}Cr release assay⁴ and *T. cruzi* using the RNA release assay described in Fig. 1. Each fraction was tested in the presence and absence of the specific antibody for the appropriate target. U, Unfractionated rat spleen; P, pellet; I, interface after Ficoll-Hypaque separation⁵; P-NA, pellet cells after passage through nylon wool (non-adherent); P-A, pellet cells recovered from the nylon wool column (adherent cells); I-NA, interface cells non-adherent to nylon wool; I-A, interface cells adherent to and recovered from nylon wool. Nylon wool was used as described previously^{6,7}. Assay against *T. cruzi* was carried out at a ratio of 20:1 for 4 h and against P815 was carried out at a ratio of 40:1 for 3 h after light centrifugation. % cytotoxicity was calculated from % isotope release: (test—control)/(maximum—control), where test = % release in the presence of antibody, control = % release in the absence of antibody, maximum = release after freezing and thawing (100% for RNA from *T. cruzi* and 81% for ^{51}Cr release from P815). The control values were 12.0% for *T. cruzi* and 15.5% from P815. None of the control values was more than 3% higher than the spontaneous release (tubes containing antibody but not effector cells). The arrow indicates a value where the difference between the presence and absence of antibody was not significant at the 5% level.

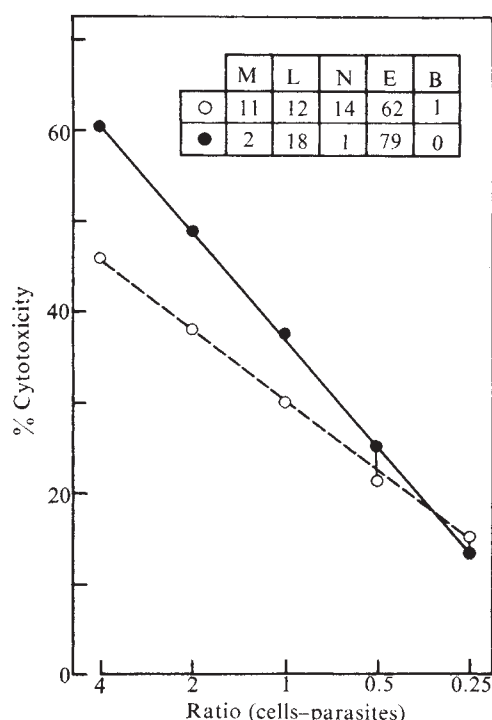


Fig. 3 An experiment using cells induced with dextran (molecular weight 70,000) and rich in eosinophils. Rats were injected with 5 ml of 6% dextran 15 h before collection of the exudate. The cells were tested unfractionated (○) and after carbonyl iron treatment (●) to remove adherent cells, by incubating 5 ml of cell suspension with 50 mg of carbonyl iron for 15 min at 37 °C. The non-adherent cells were recovered using a magnet. Differential cell counts were made after staining with May-Grunwald-Giemsa, and the values are the mean counts made by three different individuals. M, macrophages; L, lymphoid cells; N, neutrophils; E, eosinophils; B, mast cells. The two lines are significantly different at the 1% level, although the individual points joined by a vertical line are not significantly different at the 5% level. Linear regression analysis; (○) slope 5.96 ± 0.36 , correlation coefficient 0.986; (●) slope 8.99 ± 0.29 , correlation coefficient 0.996.

tumour cells. On the other hand, very little activity against *T. cruzi* is found in the low density fraction (I), which contains most of the activity against the tumour cells. The two activities are virtually mutually exclusive, as can be seen by comparing the activities in the pellet (P) with the interface cells (I) or the interface non-adherent cells (I-NA). This indicates that K cells have insignificant activity against *T. cruzi*, and that the cells active against the parasite have insignificant activity against these tumour cells. The high density of the active cells suggested that they may be granulocytes. To investigate this possibility, we induced peritoneal exudates with high proportions of granulocytes and tested them as effector cells against antibody-coated *T. cruzi*. Preliminary experiments with exudates rich in neutrophils or eosinophils suggested that eosinophils were the effector cells, and this can be seen in Fig. 3 where unfractionated exudate cells with 62% eosinophils shows spectacular activity against *T. cruzi* (a ratio of 1:1, giving 30% cytotoxicity in 4 h). Treatment of this exudate with carbonyl iron and a magnet to remove macrophages and neutrophils resulted in an increase in the activity against *T. cruzi* (a ratio of 1:1 giving 37.5% cytotoxicity). These types of experiments show that eosinophils can kill *T. cruzi*, but do not completely exclude other cell types from a subsidiary role.

In summary we have described an isotopic technique for assaying the killing of *T. cruzi* epimastigotes in which the criterion of parasite death is the release of macromolecular RNA. Apart from the obvious advantage that the assay measures the release of one of the parasites' natural macromolecules, and so can be taken as a good indicator of lysis, the fact that RNA is almost totally released when the parasites are

lysed makes the assay sensitive. We have used the assay to investigate the nature of the effector cell killing *T. cruzi* in an antibody-dependent, complement-independent system. Eosinophils show strong activity, whereas lymphoid K cells seem to have insignificant activity.

Whether this eosinophil-mediated killing plays any biological part in the pathogenesis of American trypanosomiasis remains to be tested, but like experiments with *Schistosoma mansoni*⁸, the present report adds another biological activity to our knowledge of the eosinophil.

This work was supported by grants from the Brazilian National Research Council (CNPq), and the National Fund for Research in Brazil (FINEP). CJS was supported in part by a visiting professorship from the Federal University of Rio de Janeiro. We thank Dr G. M. Oliveira Castro for his support and advice.

Note added in proof: We have been unable to induce eosinophil exudates with dextran in other strains of rats.

COLIN J. SANDERSON*
ANGEL F. LOPEZ
MARLENE M. BUNN MORENO

Instituto de Biofísica,
Instituto de Microbiologia,
Centro de Ciências da Saúde,
Universidade Federal Rio de Janeiro,
Cidade Universitária—ZC.32,
20 000—Rio de Janeiro—RJ, Brasil

Received 22 February; accepted 24 May 1977.

*On leave from the Clinical Research Center, Watford Road, Harrow, UK.

- Kierszenbaum, F. & Howard, J. C. *J. Immun.* **116**, 1208–1211 (1976).
- Mkwananzi, J. B., Franks, D. & Baker, J. R. *Nature* **259**, 403–404 (1976).
- Sanderson, C. J., Clark, I. & Taylor, G. A. *Nature* **253**, 376–377 (1975).
- Sanderson, C. J. *Proc. R. Soc. Lond. B* **192**, 221–239 (1976).
- Boyum, A. *Scand. J. clin. lab. Invest.* **21** (Suppl. 97), 77–89 (1968).
- Julius, M. H., Simpson, E. & Herzenberg, L. A. *Eur. J. Immun.* **3**, 645–649 (1973).
- Sanderson, C. J. & Thomas, J. A. *Proc. R. Soc. Lond.* (in the press).
- Butterworth, A. E. *et al. J. exp. Med.* **145**, 136–150 (1977).
- Warren, L. G. *J. Parasitol.* **46**, 529–539 (1960).
- Franks, D., Kelly, M. & Sanderson, C. J. *Int. Arch. Allerg. appl. Immun.* **48**, 621–631 (1975).

Type C virus expression and host response in diet-cured NZB/W mice

THE immune complex renal disease of NZB/W mice is largely prevented and survival of the animals markedly prolonged by protein or calorie restriction^{1,2}. These same dietary restrictions depress the antibody response^{3–7} and inhibit the development of spontaneous cancer^{8–10} in experimental animals. Spontaneous release of endogenous type C viruses (MuLV) and the autogenous host humoral immune response to these MuLV have been implicated in the aetiology of autoimmune disease in NZB and (NZB × NZW)F₁ (B/W) mice^{11–14}. We wondered whether the disease-sparing effect of a low protein diet in B/W mice was attributable to a decrease in the production of endogenous MuLV or a reduction in the host response to this virus. Our findings suggest that neither of these events is responsible for the disease prevention.

Female B/W mice, raised in our laboratory breeding colony¹⁵, were maintained from 4 weeks of age on a low phenylalanine-low tyrosine diet. This diet prolongs survival and prevents immune complex nephropathy in B/W mice¹. Control mice were fed an unrestricted *ad lib* diet. Mice were necropsied when sick or at termination of the experiment and tissues were studied by light microscopy. The low phenylalanine-low tyrosine diet led to a significant prolongation of life (Fig. 1). The median survival on this restricted diet was more than doubled, that is, 24.3 months compared to 9.7 months for mice on the *ad lib* diet, and the relative death rate¹⁶ was nearly fivefold less than in the *ad lib* diet group ($P < 0.001$). Of 23 mice in the restricted diet group, 15 were killed while still healthy at 2 yr

Table 1 Type C virus recovery from tissues cultivated from female B/W mice on amino acid restricted and *ad lib* diets

Diet	Xenotropic	Virus isolations		General titre (PFU ml ⁻¹)
		General titre (FFU ml ⁻¹)	Ecotropic	
Low amino acid	3/3	1,000	1/3	10,000
<i>Ad lib</i>	5/5	1,000	2/5	10,000

Values represent the number of mice which yielded virus/number tested. Assay for X-tropic virus in B/W mice was performed by initially co-cultivating mouse tissues with nonvirus producing murine sarcoma virus (MSV) transformed rat cells. Spleen, thymus and kidney tissues were co-cultivated from each mouse. After 7 d, the supernatant of these cultures was filtered and assayed for focus forming virus on rat or human cells^{17,18}. Titre is expressed as focus forming units (FFU) per ml and is the general titre observed with cultures of the individual organs. The quantity of X-tropic virus produced by the mouse cells correlated with the amount of pseudotype MSV produced by co-cultivation²⁷. Ecotropic virus produced by the mouse tissues was assayed by the standard XC plaque technique²⁸. The titre is expressed as plaque forming units (PFU) per ml.

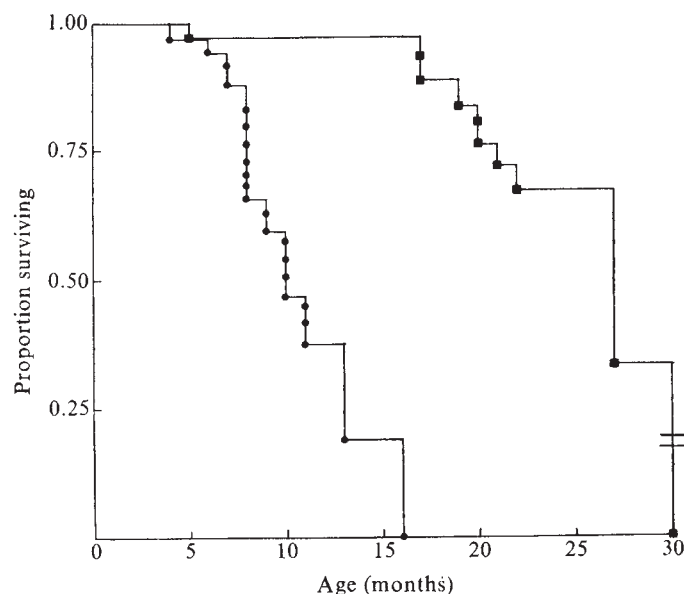


Fig. 1 Survival curves of B/W female mice on amino acid restricted (■) and *ad lib* (●) diets. The amino acid restricted group contained 35 mice and the *ad lib* diet group 32 mice. The data include killed and dead mice under long term observation as well as those sacrificed at 8 months of age. The survival curves and relative death rates were calculated using the actuarial life table approach and observed numbers of death in each group were compared to their expected numbers, assuming that no difference existed between groups¹⁶.

of age and minimal glomerulonephritis was observed in only 3. Of the 8 mice in this diet group that were found dead between 5 and 30 months of age, 3 showed moderate glomerulonephritis and the other 5 died of unrelated causes. Thus, about 75% (17 of 23) of the diet-restricted mice were free of renal disease at an average age of 20 months. In the *ad lib* group, by contrast, all (29 of 29) mice had severe glomerulonephritis at an average age of 8 months. In general, the glomeruli in the diet-restricted group were neither enlarged nor hypercellular and there was no thickening of the mesangial matrix or capillary basement membranes. Immunoperoxidase staining also demonstrated a marked decrease of immunoglobulin deposition in the kidneys of the restricted diet mice compared to the control mice (data not shown). We thus confirmed that diet-restriction has a profound inhibitory effect on the development of immune complex (IC) nephritis in B/W female mice ($P < 0.001$). A similar preventive effect upon malignancy was not noted since lymphomas were found in two of the amino-acid restricted B/W mice at 21 and 24 months of age, whereas no lymphomas were found in the shorter lived B/W mice on the *ad lib* diet.

To determine whether the diet had any influence on production of endogenous type C virus we assayed tissues of amino acid-restricted B/W females aged 19–23 months and compared their virus expression with that of B/W controls on

an *ad lib* diet. It has been shown that all cells from NZB and B/W mice produce in substantial titres an endogenous xenotropic (X-tropic) virus that can productively infect only cells from a heterologous species^{13,14,17,18}. Tissues from the B/W hybrid sometimes also produce an endogenous ecotropic virus, present in the NZW parent, which infects and spreads readily in mouse cells¹³. As shown in Table 1, the prevalence and titre of X-tropic virus released by different tissues of the amino acid-restricted B/W mice were equivalent to those of control B/W mice. It is apparent that the dietary prevention of IC nephritis in B/W mice was not linked to any decrease in the production of endogenous X-tropic virus as measured by our *in vitro* technique. Moreover, since these diet-restricted mice had almost no signs of IC nephritis 1–11 months after the virus titre in their littermates was assessed, high production of X-tropic virus did not correlate with ultimate development of kidney lesions. In two controls and one experimental mouse, ecotropic AKR-MuLV was also detected. The presence of this virus was not associated with any specific pathological changes in the kidney.

We next determined whether the prevention of IC nephritis by the restricted diet correlated with any reduction in the host

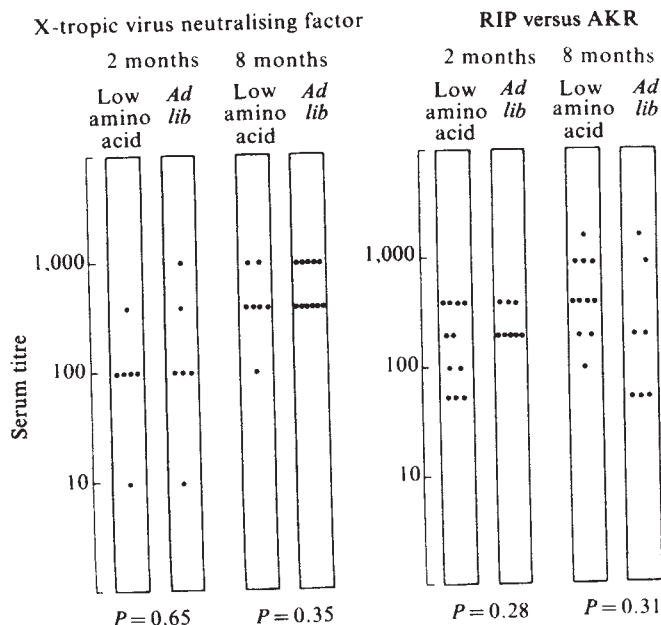


Fig. 2 Levels of anti-viral activity in female B/W mice fed amino acid restricted and *ad lib* diets. Sera were diluted 1:5 in phosphate buffered saline. Neutralisation activity was measured by reduction of foci on NRK cells of an NZB pseudotype sarcoma virus bearing the X-tropic virus type specific envelope antigen, as described^{18,23}. The radioimmune precipitation assay of intact labelled ecotropic (AKR) virus has also been described²⁰. At 8 months of age severe glomerulonephritis was present in all controls but was totally absent in those mice fed the restricted diet.

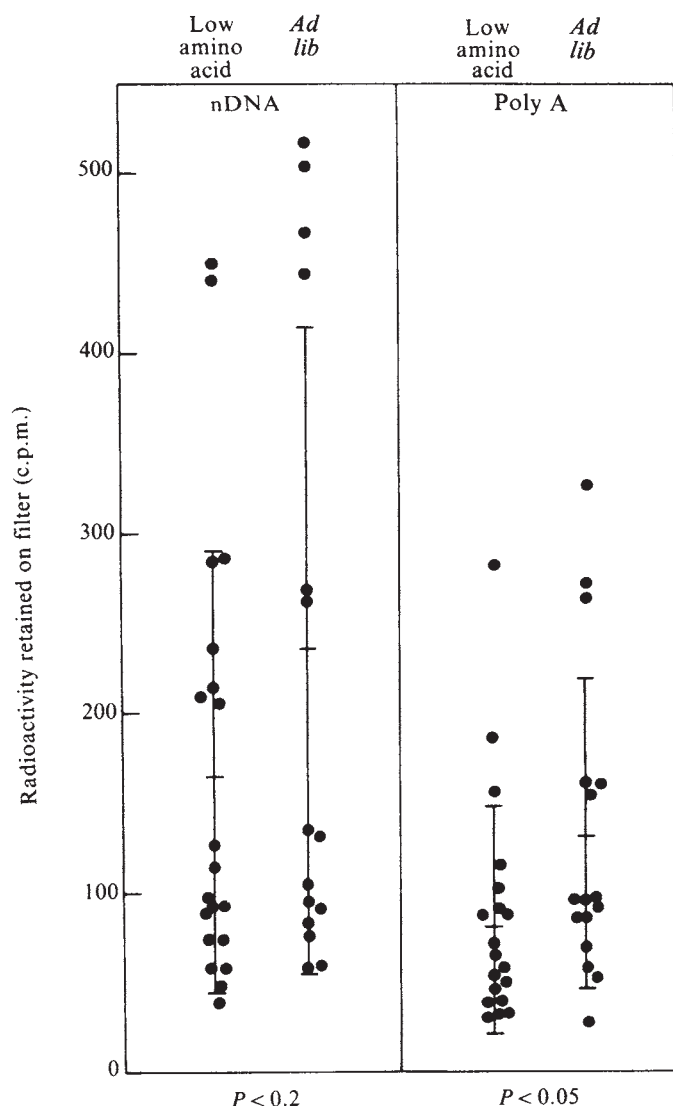


Fig. 3 Serum anti-nucleic acid antibodies in 8 month old female B/W white mice fed amino acid restricted and *ad lib* diets. Antibodies to native DNA (nDNA) and polyribonucleic acid (poly A), a single stranded RNA, were determined by a cellulose ester filter radioimmunoassay²⁹. 100 μ l of a 1:10 dilution of serum in minimum essential medium was heat inactivated at 56 °C. After adding 10 μ l of ³H-DNA (116 ng, 800 c.p.m.) or ³H-poly A (7 ng, 800 c.p.m.), the reaction mixtures were incubated at 37 °C followed by incubation for 18 h at 0 °C. The reaction mixtures were diluted and passed through Millipore filters. The filters were washed, dried, and the radioactivity retained on the filters was counted by liquid scintillation.

response to endogenous viruses. A breakdown in the control of endogenous type C viruses could lead to high tissue and serum concentration of these viruses and the resultant hyper-response to one or more of the viral antigens, for example, envelope glycoproteins (gp70), might contribute to the renal glomerular immune complex deposition^{11,19}. Binding antibodies to intact labelled AKR ecotropic virus have been found in the sera of B/W mice²⁰ but no antibodies to X-tropic virus have been detected²¹. But, a serum non-immunoglobulin factor associated with mouse lipoproteins has been identified which neutralises specifically this X-tropic class of endogenous MuLV (refs 22, 23). No significant differences were found in the titre of either X-tropic virus neutralising factor or antibodies to the AKR-MuLV in sera from the mice in amino acid-restricted or *ad lib* diet groups (Fig. 2). In both diet groups the X-tropic virus neutralisation titres increased somewhat with age. A similar age increase in the AKR precipitating

antibody titre was noted in the amino acid restricted but not in the *ad lib* group. In general, the geometric mean titres (g.m.t.) and the titre range of both X-tropic virus neutralising factor (g.m.t. 500, range 100–1,000) and anti-ecotropic virus antibody (g.m.t. 320, range 100–1,600) were similar in both diet groups at 8 months of age to those values previously detected in untreated NZB and B/W mice^{14,20,21}. Although subtle differences might yet be revealed if these analyses were done on a larger number of mice, these findings do suggest that the diet-induced prevention of immune complex nephritis in B/W mice is not due to any major decrease in production of X-tropic virus neutralising factor or precipitating antibodies to the endogenous ecotropic virus.

Another characteristic of the autoimmune disease complex in B/W mice is antibodies to nucleic acids. We found the mean binding of serum antibodies to native DNA and poly A was somewhat lower at 8 months of age in the group fed the amino acid-restricted diet compared to the control group (Fig. 3); this reached statistical significance ($P < 0.05$) only to poly A.

These data suggest that the dietary-induced increase in longevity and prevention of immune complex disease in female B/W mice is unrelated to any influence upon production of endogenous type C viruses or the autogenous host response to these viruses. A similar lack of association between autoimmune markers and high titres of xenotropic virus was found in F₁ progeny of crosses between NZB mice and other strains of laboratory mice²⁵. If one assumes that virus production *in vivo* is accurately represented by the *in vitro* virus assays, these observations suggest that the IC renal disease in B/W mice is not solely a consequence of spontaneous oncornavirus production. The trend towards reduction in antibodies to nucleic acids observed in the diet-treated mice, although not profound, may have contributed to the reduction in IC nephritis since about half of the immunoglobulin deposited in glomeruli of control B/W mice reacts with nucleoprotein²⁶. Measurement of the total immunoglobulin levels, the types of immunoglobulins produced, and the quantitation of circulating immune complexes might clarify further whether the diet-induced disease-protection involves a generalised decrease in antibody synthesis. The diet may decrease certain species of immunoglobulins or complexes other than those containing viral or nuclear antigens. These studies suggest, however, that since the diet did not significantly affect the host response to endogenous viruses or nucleic acids, other factors such as hormone levels and the number and function of cells responsible for mediating the immunological reactions may have been affected.

We thank Drs Robert Huebner and Frank Dixon for critical review, Ms Patricia Kazan and Mr John Estes for technical assistance, Dr Clive Taylor and Mr Robert Rongey for immunoperoxidase studies and Dr Malcolm Pike and Mr John Casagrande for statistical analyses. This work was supported by a contract (NO1 CP 53500) and a grant (USPHS CA 13086) from the US NCI. J.A.L. is the recipient of a Research Career Development Award (N05 KA4 CA 70990) from the NCI.

M. B. GARDNER

Department of Pathology,
University of Southern California,
School of Medicine,
Los Angeles, California 90033

J. N. IHLE

Basic Research Program,
Frederick Cancer Research Center,
Frederick, Maryland 21701

R. J. PILLARISSETTY
N. TALAL

Immunology-Arthritis Section,
Department of Medicine, University of California,
Veterans Administration Hospital,
San Francisco, California

E. L. DUBOIS

*Rheumatic Disease and Immunology Section,
Department of Medicine, University of Southern California,
School of Medicine, Los Angeles, California 90033*

J. A. LEVY

*Department of Medicine and Cancer Research Institute,
University of California,
San Francisco, California 94122*

Received 7 January; accepted 31 May 1977.

- ¹ Dubois, E. & Strain, L. *Biochem. Med.* **7**, 336–342 (1973).
- ² Fernandes, G., Yunis, E. J. & Good, R. A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1279–1283 (1976).
- ³ Cooper, W. C., Good, R. A. & Mariani, T. *Am. J. clin. Nutr.* **27**, 647–664 (1974).
- ⁴ Aschkenasy, A. *Nature* **250**, 325–327 (1974).
- ⁵ Aschkenasy, A. *Nature* **254**, 63–65 (1975).
- ⁶ Fernandes, G., Yunis, E. J. & Good, R. A. *J. Immun.* **116**, 782–790 (1976).
- ⁷ Coovadia, H. M. & Soothill, J. F. *Clin. exp. Immun.* **23**, 562–567 (1976).
- ⁸ Saxton, J. A., Boon, M. C. & Furth, J. *Cancer Res.* **4**, 401–409 (1944).
- ⁹ Fernandes, G., Yunis, E. J. & Good, R. A. *Nature* **263**, 504–507 (1976).
- ¹⁰ Heston, W. E. & Vlahakis, G. J. *natn. Cancer Inst.* **27**, 1189–1196 (1961).
- ¹¹ Mellors, R. C., Shirai, T., Aoki, T., Huebner, R. J. & Krawczynski, K. J. *exp. Med.* **133**, 113–132 (1971).
- ¹² Mellors, R. C., Aoki, T. & Huebner, R. J. *J. exp. Med.* **129**, 1045–1062 (1969).
- ¹³ Levy, J. A. *Am. J. clin. Path.* **62**, 258–280 (1974).
- ¹⁴ Levy, J. A. *Rheumatology* **2**, 135–148 (1975).
- ¹⁵ Dubois, E. L., Horowitz, R. E., Demopoulos, H. B. & Teplitz, R. J. *Am. med. Ass.* **195**, 285–289 (1966).
- ¹⁶ Peto, R. *Br. J. Cancer* **29**, 101–105 (1975).
- ¹⁷ Levy, J. A. & Pincus, T. *Science* **170**, 326–327 (1970).
- ¹⁸ Levy, J. A. *Science* **182**, 1151–1153 (1973).
- ¹⁹ Yosikuni, T., Mellors, R. C., Strand, M. & August, J. T. *J. exp. Med.* **140**, 1011–1027 (1974).
- ²⁰ Lee, J. C. & Ihle, J. N. *J. natn. Cancer Inst.* **55**, 831–838 (1975).
- ²¹ Strand, M. & August, J. T. *Virology* **75**, 130–144 (1976).
- ²² Leong, J., Kane, J., Oleszko, O. & Levy, J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 276–280 (1977).
- ²³ Levy, J. A., Ihle, J. N., Oleszko, O. & Barnes, R. D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5071–5075 (1975).
- ²⁴ Nowinski, R. C. & Kaehler, S. L. *Science* **185**, 869–871 (1974).
- ²⁵ Datta, S. K. & Schwartz, R. S. *Nature* **263**, 412–414 (1976).
- ²⁶ Dixon, F. J., Oldstone, M. B. A. & Toniotti, G. J. *exp. Med.* **134**, 65s–71s (1971).
- ²⁷ Levy, J. A., Kazan, P., Varnier, O. & Kleiman, H. J. *Viol.* **16**, 844–853 (1975).
- ²⁸ Rowe, W. P., Pugh, W. E. & Hartley, J. W. *Virology* **42**, 1136–1139 (1970).
- ²⁹ Talal, N., Pillaristetty, R. J., DeHoratius, R. J. & Messner, R. *Clin. exp. Immun.* **25**, 377–382 (1976).

Epizootiology, transmission and approach to prevention of fatal simian haemorrhagic fever in rhesus monkeys

RHESUS monkeys (*Macaca mulatta*) and all other macaques are extremely susceptible to the fatal disease, simian haemorrhagic fever (SHF). Introduction of this agent into a laboratory colony will result rapidly in the complete loss of all macaques in that colony^{1–6}. An outbreak in one of our rhesus colonies resulted in the loss of all 212 animals in a period of 12 d. Because of this great loss and its consequences, we initiated an investigation into the epizootiology, transmission and prevention of this disease. These studies have shown that the virus is carried as a chronic infection in patas monkeys (*Erythrocebus patas*) with no apparent signs of disease. It can be transmitted to the rhesus monkeys by accidental inoculation with tissue fluids, and once they are infected, spread by aerosol and contact is rapid.

Clinically, SHF is a febrile, haemorrhagic disease of macaques caused by an RNA virus which has been reported to be a togavirus⁵. The disease is characterised by rapid onset, early fever, mild facial oedema, failure to eat or drink, dehydration, protein in the urine, cyanosis, haemorrhages of the skin, bloody diarrhoea, nosebleeds, occasional bleeding in the orbit of the eye and subsequent death in most cases. Pathological changes associated with the disease include widespread small to large skin haemorrhages, haemorrhagic tissue death of the first few centimetres of the small intestine, enlarged spleen, intravascular coagulation and generalised destruction of lymphoid tissue^{3,7}.

The epizootiology of SHF has been described for eight outbreaks^{1,2,6}. The disease has only been observed in macaques. The first seven outbreaks involved recently imported rhesus monkeys with subsequent spread of the disease to other macaque species. The source of the eighth outbreak was not identified. All of the epizootics were

characterised by an explosive onset, rapid spread within exposed groups and a high case-fatality ratio. No human illness was associated with either the current outbreak or previously reported epizootics of SHF.

In November 1972, an outbreak of SHF occurred in our colony in a building housing two groups of monkeys: 212 rhesus monkeys and 46 patas monkeys. The two groups were kept in separate rooms connected by an open loft: no other animals were housed in the building. Only the rhesus monkeys were involved and of the 212 animals, 172 had been in the colony for 6 months to 1 yr. The other 40 rhesus monkeys were introduced into the colony in a one-week period. The new animals had been conditioned for 60 d at the facilities of an animal importer, during which they were held in two separate rooms with no other animals. The patas monkeys had been in our colony for 2 yr.

Except for diarrhoeal disease in 10 of the newly arrived rhesus monkeys, no major disease problems were noted in the colony until about 30 d after the last animal was introduced. Suddenly, within one day, 60 rhesus monkeys, including all 40 newly arrived animals, developed a clinical illness characterised by depression and loss of appetite. The following day, an increasing number developed a similar illness. Many of the animals observed to be ill the previous day had developed facial cyanosis and showed dilated pupils; 15 of the animals were too weak to stand. The first fatalities occurred 3 d after the onset of illness, when 10 animals were found dead. Within 4 d, all remaining rhesus monkeys were affected clinically, and nine additional monkeys had died. In 12 d, a total of 106 monkeys had died. At this time, all surviving macaques were in advanced stages of illness and so were killed. At necropsy the affected animals had lesions characteristic of SHF. At no time did the patas monkeys exhibit signs of disease.

The disease seemed to have spread within the rhesus colony by direct and indirect contact exposure. Although all monkeys in the colony were in individual cages separated by a solid metal partition, they could touch each other by reaching around the partition. Also, all the female rhesus monkeys were weighed twice weekly as part of a nutritional experiment. For this, the animals were placed in one of three portable cages for transport to the scale. The portable cages were washed and autoclaved at the end of each day. Indirect contact of the monkeys did, however, result during use of these transfer cages for several animals on a given day. On some occasions, the same cages were also used to transport the patas monkeys to the scale.

Although spread by means of needles used in the treatment and tattooing of monkeys had been incriminated in previous epizootics of SHF, exposure by this route in this outbreak was investigated carefully and found to be limited to a small number of animals. The 40 newly arrived rhesus monkeys were tattooed using the same equipment on or shortly after arrival. The tattoo needle was cleaned with alcohol between animals. A few animals in the newly arrived group may have been given injections of vitamins and antibiotics with a common needle. Most of the infected animals, however, had not been tattooed or inoculated for a number of months before the outbreak.

In this outbreak, approximately 90 d (60 d at importer's and 30 d in our facility) had elapsed between importation of the newly introduced group of monkeys and the onset of the epizootic. Disease compatible with SHF had not been noted since 1965 at the animal importer's facilities and the importer had not heard of any similar disease problem in monkeys sold to other customers. In experimental studies the incubation period of SHF has ranged from 3–7 d after inoculation, with most deaths occurring within 7–13 d (refs 2 and 7). The short incubation period of the disease, the long time which elapsed between importation of the newly introduced monkeys and the onset of the epizootic, and absence

of the disease in animals at the importer's facilities suggest that the rhesus monkeys were first exposed to the disease at the NIH colony. Studies on the transmission of the SHF were initiated and demonstrated that the disease could be spread from infected to uninfected rhesus monkeys by direct contact with animals in the same cage or by aerosol to animals in adjacent cages, as well as by inoculation. With inoculation, the animals died in 7–12 d.

In view of the extreme susceptibility and lethality of SHF in rhesus monkeys it is likely that this is not a natural epizootic disease of macaques. Once introduced into macaques either in the wild or laboratory colony, the disease would destroy essentially all animals in two weeks. The few remaining animals seen in laboratory conditions following a long period of supportive treatment are not chronic carriers of the virus, but have high levels of antibodies⁴.

The investigation of the possible source and method of introduction of the infection into our rhesus population was fortunately made easier because only one other species of sub-human primate was in the building. For this reason, careful study of the patas monkeys was also initiated to determine if they were the source of the infection. Tests showed that when serum from some patas was inoculated intravenously into a rhesus monkey, SHF and death resulted in 4 d. In a subsequent series of studies, it was found that approximately 10% of recently imported patas monkeys were chronic carriers of SHF. Minute quantities of blood or tissue fluids could transmit the infection to the rhesus. Transmission did not occur between patas and rhesus when the animals were housed together in the same cages, so it obviously required direct mechanical transmission of infected tissues and did not occur by contact or aerosol. It seems that our epizootic was initiated by the use of a multidose vial which was used to inoculate animals of both species. The inappropriate re-introduction of a contaminated needle from animals with more than 10^{-12} ID (infective doses) per ml of blood into the vial following the injection of a chronically infected patas would have been sufficient to contaminate the vial contents and spread the disease to rhesus monkeys which were subsequently infected with material from the same vial.

Prevention of SHF can be accomplished by interrupting transmission from the natural reservoirs of infection. Transmission of SHF on this occasion occurred because asymptomatic patas monkeys, which chronically carry this virus, were maintained in the same building by the same personnel as rhesus monkeys and, unfortunately, because of a technical error, some of the contaminated tissue fluids from such a patas monkey was inoculated into a rhesus. Once the rhesus animal was infected, the disease spread through the rhesus colony by direct contact and aerosol. Thus, prevention of SHF in such colonies must include meticulous attention to prevent procedures which might transmit infected tissues from patas to rhesus. Whenever possible, the two species should be housed in separate facilities to minimise further the risk of cross inoculation.

Patas animals can carry this deadly virus for at least 2 yr, and we have also found that approximately 1% of baboons (*Papio papio*) and African green monkeys (*Cercopithecus aethiops*) we have studied carried latent SHF. To date, there has been no evidence that chimpanzees carry this virus. All other African species, however, should be considered possible carriers. Importations and shipments of monkeys must also be arranged to separate the African species from macaques since inadvertent exposure to blood or tissue fluid might occur in open wounds during shipment. Since SHF is a togavirus, the possibility of arthropod-borne transmission must also be investigated.

We have also reported that the complex of polyriboinosinic-polyribocytidylic acid-containing poly-L-lysine and carboxymethylcellulose is effective in preventing the spread

of SHF in colonies of macaques if used in recently exposed animals⁵. Animals showing signs of disease however, were not helped by using this drug.

WILLIAM T. LONDON

Infectious Diseases Branch, IRP,
National Institute of Neurological and
Communicative Disorders and Strokes,
National Institutes of Health,
Bethesda, Maryland 20014

Received 23 March; accepted 23 May 1977.

- 1 Shevtosva, A. V. *Vop Virusol.* 12, 47–51 (1967).
- 2 Palmer, A. E., Allen, A. M., Tauraso, N. M. & Shelokov, A. *Am. J. trop. Med. Hyg.* 17, 404–412 (1968).
- 3 Allen, A. M., Palmer, A. E., Tauraso, N. M. & Shelokov, A. *Am. J. trop. Med. Hyg.* 17, 413–421 (1968).
- 4 Tauraso, N. M., Shelokov, A., Palmer, A. E. & Allen, A. M. *Am. J. trop. Med. Hyg.* 17, 422–431 (1968).
- 5 Trousdale, M. D., Trent, D. W. & Shelokov, A. *Proc. Soc. exp. Biol. Med.* 150, 707–711 (1975).
- 6 Espana, C. *Lab. Anim. Sci.* 21, 1023–1031 (1971).
- 7 Abildgaard, C., Harrison, J., Espana, C., Spangler, W. & Gribble, D. *Am. J. trop. Med. Hyg.* 24, 537–544 (1975).
- 8 Levy, H. G., London, W., Fuccillo, D. A., Baron, S. & Rice, J. J. *infect. Dis.* 133, A256–A259 (1976).

Y-forms as possible intermediates in the replication of measles virus nucleocapsids

THE genome of measles virus, a paramyxovirus, is a non-segmented single stranded RNA molecule which is enclosed within a helix of protein subunits to form a linear nucleocapsid¹. Little is known about its mode of replication. It is known, however, for other paramyxoviruses that during the growth cycle transcription of the RNA occurs from an intact nucleocapsid by a virion-associated enzyme^{2–4}. The resulting messenger RNA molecules are less than the full genome length and are unencapsidated⁵. By contrast, during viral RNA replication no free genome length RNA can be detected^{5,6} and both plus and minus-stranded RNA are found only in the encapsidated form⁷. From this evidence, Kingsbury has suggested⁷ that nucleocapsids of paramyxoviruses replicate without separation of the protein and nucleic acid portions of the template nucleocapsid and has implied that plus and minus-stranded RNAs enclosed within the capsid structure act as templates for each other. If this is so, association of the two types of nucleocapsid in a replicative form should be detectable in infected cells. We have conducted a search by electron microscopy for such structures in measles virus infected cells and the results are described here.

Vero cell monolayer cultures were infected with the P9 strain of measles virus⁸ at a multiplicity of 1 pfu cell⁻¹. Preparations of nucleocapsid material for electron microscopy were obtained by lysis of the monolayers with a few drops of 2% phosphotungstic acid at 18–24 h post-infection, the time of maximum nucleocapsid synthesis. A small drop of the lysate was applied to a carbon-Formvar coated grid, blotted dry with filter paper and viewed in the electron microscope. Nucleocapsid lengths were measured on tracings of enlarged electron micrograph negatives or by counting capsid helix turns.

The majority of nucleocapsids observed were linear molecules. However, rare Y-shaped structures with two arms of equal and variable length were also observed (Fig. 1). Such structures would be expected for a replication mode in which one strand was being copied from one end. The variable lengths of the arms indicate that the structures represent different stages of replication.

From a large number of experiments, 26 structures were selected which we considered to be unambiguous Y-forms. In those with very short arms (Fig. 1a, b), the number of turns of the capsid helix was the same, ± 1 , for both arms. In those with longer arms, the lengths differed only by an amount probably consistent with the deformations introduced during preparation. The total length of the 'unreplicated' stem portion

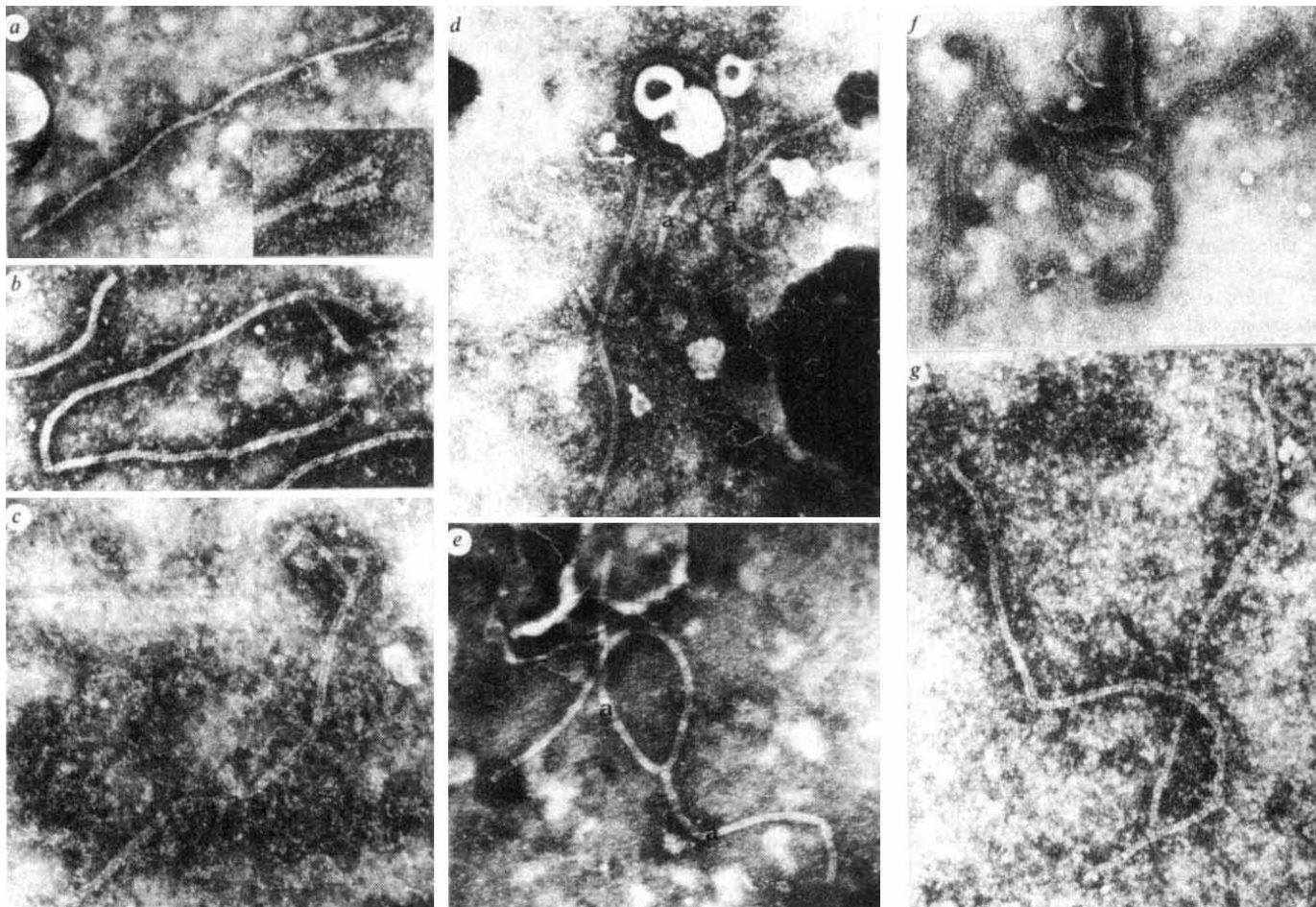


Fig. 1 Y-form nucleocapsid at various stages of replication. Lengths of arms and stems, respectively. *a* $\times 39,600$ (inset $\times 99,000$). 0.07 μm , 0.07 μm and 1.45 μm . Nine turns of capsid helix on each arm. *b* $\times 66,000$. 0.12 μm (21 turns), 0.13 μm (22 turns) and 1.34 μm . *c* $\times 92,400$. 0.18 μm , 0.16 μm and 0.7 μm . *d* $\times 66,000$. 0.73 μm , 0.73 μm and 0.6 μm . Arrow indicates junction of arms (*a*) and stem. *e* $\times 66,000$. 0.5 μm , 0.53 μm and 0.8 μm . *f* $\times 66,000$. 0.34 μm , 0.33 μm and 1.21 μm . *g* $\times 66,000$. 1.26 μm , 1.07 μm and 0.13 μm .

plus one arm was between 0.8 and 1.6 μm . This is similar to the range of lengths found for non-replicating nucleocapsids in the same preparations. The variation in length of the 'unreplicated' portion of the Y-forms was apparently random. Molecules adventitiously joined in suspension or on drying on the grid can generally be distinguished by the overlapped appearance of the junction and by the lack of relationship between the lengths of the three arms.

A feature apparent in most (22 out of 26) of the 'replicating forms' is that the capsomeres in one arm of the Y-forms have the opposite orientation to those in the other (Fig. 1*a, b, f, g*). This might be expected from the presumed opposing polarities of the encapsidated complementary RNA chains. If, however, one of the arms had turned over, the orientation of capsomeres would be the same in both arms (Fig. 1*d, e*). Assuming that the RNA is replicated by presently known processes, the new chain beginning with the 5' end would be copied from the 3' end of the parent molecule. By reference to Fig. 1*b*, it can be seen that this allows identification of the 3' end of the nucleocapsid as the end with the convex orientation of capsomeres.

The structural features at the junction are not yet apparent although some alteration of capsid arrangement is suggested by Fig. 1*a, b* and *g*. Better preserved preparations and higher resolution are required for further study.

Rigid proof that the Y-forms represent the true replicative form may be difficult to obtain since they occur only at very low frequency, presumably owing to the rapid rate of replication. Our attempts to increase the number of Y-forms by arrest of nucleocapsid synthesis by rapid freezing and by the use of inhibitors have so far been unsuccessful. These preliminary

results, however, support the idea that the nucleocapsid is replicated from an intact nucleocapsid template with local unwinding to expose the enclosed RNA and rapid assembly of progeny nucleic acid and protein⁷.

We previously reported the presence of circular nucleocapsid molecules in measles-infected cells⁹. Similar circular molecules, which were thought to be end-to-end aggregates of linear nucleocapsid, had been already reported for another paramyxovirus, NDV, by Compans and Chopin¹⁰ but we were unaware of their work. It seems likely from the results presented here that circular nucleocapsids do not function in the replication of the normal linear nucleocapsid population. We have, however, found rare circular molecules which appear to have attached tails in even lower proportion than the Y-forms. These may represent templates functioning in a rolling-circle type of mechanism as previously suggested⁹ but this requires further study.

This work was supported by an MRC Programme grant. We thank Mr G. Clarke and Mr T. McLaughlin for assistance.

H. V. THORNE
EVELYN DERMOTT

*Department of Microbiology and Immunobiology,
The Queen's University of Belfast,
Grosvenor Road, Belfast,
Northern Ireland*

Received 22 April; accepted 24 May 1977.

- 1 Finch, J. T. & Gibbs, A. J. *J. gen. Virol.* 6, 141-150 (1970).
- 2 Huang, A. S., Baltimore, D. & Bratt, M. A. *J. Virol.* 7, 389-394 (1971).

- ³ Robinson, W. S. *J. Virol.* 8, 81-86 (1971).
⁴ Stone, H. O., Portner, A. & Kingsbury, D. W. *J. Virol.* 8, 174-180 (1971).
⁵ Blair, C. D. & Robinson, W. S. *J. Virol.* 5, 639-650 (1970).
⁶ Robinson, W. S. *Virology* 44, 494-502 (1971).
⁷ Kingsbury, D. W. *Med. microbiol. Immunol.* 160, 73-83 (1974).
⁸ Shirodaria, P. V., Dermott, E. & Gould, E. A. *J. gen. Virol.* 33, 107-115 (1976).
⁹ Thorne, H. V. & Dermott, E. *Nature* 264, 473-474 (1976).
¹⁰ Compans, R. W. & Choppin, P. W. *Virology* 33, 344-346 (1967).

Presence of 1,25-dihydroxyvitamin D₃-glycoside in the calcinogenic plant *Cestrum diurnum*

A DISEASE clinically and morphologically similar to vitamin D-intoxication, characterised by hypercalcaemia and extensive soft tissue calcification, has been described in grazing animals and linked to the ingestion of leaves from several plant species including *Cestrum diurnum* (*C.d.*)¹⁻³ and *Solanum malacoxylon*³. Early reports indicated that activity resembling the hormonal form of vitamin D₃, 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃), existed in extracts of both of these plants³⁻⁵. The active calcitropic principle from *S. malacoxylon* has been identified as a 1,25-(OH)₂D₃-glycoside⁶⁻⁸. We report here the isolation and identification of a 1,25-(OH)₂D₃-glycoside in *C.d.* It is proposed that this principle accounts for the hyperabsorption of calcium found in animals eating this plant.

We have previously reported that the ingestion of *C.d.* results in increased calcium absorption and induced calcium binding protein (CaBP) synthesis in chicks fed a diet containing strontium⁵. Strontium blocks the renal 1-hydroxylase enzyme which converts vitamin D₃ to active 1-hydroxylated metabolites⁹. Hence, any plasma 1-hydroxylated-D-vitamins in these animals must originate exogenously. With the development of a specific radioreceptor assay for 1,25-(OH)₂D₃ (refs 10-12), it is now possible to directly assess the 1,25-(OH)₂D₃ activity in the plasma of chicks after *C.d.* administration. Table 1 reveals that after a single oral dose of *C.d.*, there is a rapid increase in plasma 1,25-(OH)₂D₃-like radioreceptor activity followed by an elevation in duodenal CaBP. Within 12 h the 1,25-(OH)₂D₃-like activity increases about eightfold over the normal physiological concentration of the hormone in chicks (5.2 ng dl⁻¹) (ref. 14). These observations suggest that the biologically active calcaemic principle of *C.d.* might be 1-hydroxylated in the native plant, since the 1-hydroxyl moiety of vitamin D analogues has been shown to be essential for efficient binding to the chick intestinal receptor protein used in the radioreceptor assay. Consequently, when this calcaemic plant factor is ingested by animals, it might circumvent the stringent renal regulation of the circulating level of the 1,25-(OH)₂D₃ hormone.

Celite liquid-liquid partition chromatography¹² was then used to analyse plasma from chicks treated with *C.d.* Aliquots of plasma obtained from the experiment described in Table 1 were combined with tracer radioactive 1,25-(OH)₂D₃ and

chromatographed on Celite (Fig. 1a). The coincident migration of authentic hormone with the radioreceptor activity from the chick plasma further substantiates that *C.d.* must contain a factor very similar, if not identical, to 1,25-(OH)₂D₃.

Contrary to this idea that the plant factor is identical to 1,25-(OH)₂D₃ is the slightly slower observed appearance of intestinal CaBP after administration of *C.d.* as compared with that observed following administration of the pure hormone¹⁵. Also, we could not demonstrate the receptor activity for the crude *C.d.* extract that was predicted by bioassay of this material⁵. These observations suggested that some preliminary modification of the *C.d.* principle might be required in the animal before receptor binding and consequent biological activity. We have previously shown that hydrolysis of the

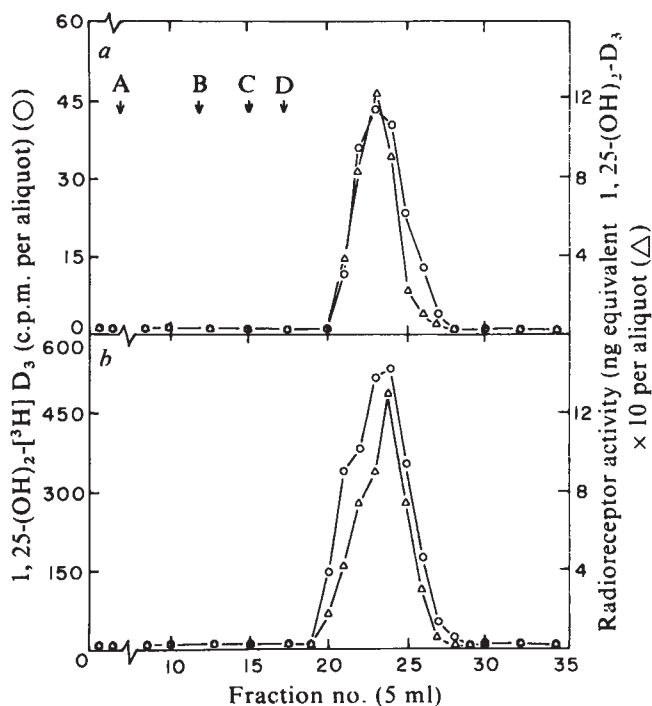


Fig. 1 Comigration on Celite of the lipid soluble 1,25-(OH)₂D₃-like factor from *C.d.* with authentic tritiated hormone. *a*, After an oral dose of *C.d.*, chick plasma was extracted with CHCl₃:CH₃OH (1:2) and partially purified via Sephadex LH-20, silicic acid, and microCelite chromatography¹². The 1,25-(OH)₂D₃-like material from *C.d.* co-eluted with standard, tritiated 1,25-(OH)₂D₃ (5500 c.p.m., 0.32 ng) in each of these column systems (data not shown). The material was then subjected to a long Celite column (1 × 40 cm) (*a*) and the radioreceptor activity was compared to the migration position of the 1,25-(OH)₂[³H]D₃ internal marker. Other vitamin D forms migrate as indicated: A, 25-hydroxyvitamin D₃; B, 24,25-dihydroxyvitamin D₃; C, 25,26-dihydroxyvitamin D₃; D, 1,25-dihydroxyvitamin D₂ (ref. 12). *b*, Dried *C.d.* leaf (300 g) was extracted with CHCl₃ (discarded) and the nonsoluble material was further extracted with CHCl₃:CH₃OH (1:2). The active material was precipitated with acetone and the pellet subsequently redissolved in CHCl₃:CH₃OH (1:2). After removal of this solvent, the material was suspended in citrate-phosphate buffer (pH 5.0) and 500 mg of mixed glycosidases derived from the liver of the sea worm, *Charonia lampas* (Miles), was added. The mixture was incubated for 16 h at 37 °C under N₂ and the material was placed into a separatory funnel with H₂O:CH₃OH:CHCl₃ (1:1:1.5) and the phases allowed to separate. The lower CHCl₃ phase was then combined with three CHCl₃ washes of the upper phase and dried in a rotary evaporator under N₂ in a vacuum. Bioassay identification of the active fraction was carried out by administration to strontium-fed chicks and measuring intestinal calcium absorption and calcium binding protein⁵. Tracer 1,25-(OH)₂[³H]D₃ (15,000 c.p.m., 0.875 ng) was added to the dried, hydrolysed material and the mixture was pre-purified by silicic acid chromatography (1 × 20 cm) eluted with ether: acetone (95:5 v/v) under N₂ pressure. The active radioreceptor fractions were combined and purified by Sephadex LH-20, microCelite chromatography and, finally (*b*) a 1 × 40 cm Celite liquid-liquid partition column¹².

Table 1 Appearance of plasma 1,25-(OH)₂D₃-like activity and intestinal calcium binding protein (CaBP) in chicks given an oral dose of *C. diurnum*

Time after dose (h)	Plasma radioreceptor activity (ng equivalent 1,25-(OH) ₂ D ₃ per 100 ml)	Intestinal CaBP (μg per mg protein)
0	<0.5	0
6	24	<0.3
12	43	1.4
24	18	6.9

White leghorn cockerels were placed on a diet⁵ supplemented with 1.2 IU vitamin D₃ per g, 2.56% Sr, 0.05% Ca and 0.76% P. Each chick received an oral dose of *C.d.* extract equivalent to 2 g original dried leaf. Plasma from five chicks was pooled, purified, and assayed for 1,25-(OH)₂D₃-like receptor activity as previously described¹²; data represent the mean of triplicate assays for each pooled sample. Duodenal CaBP was quantitated by radial-immunoassay¹³ in supernatant fractions of homogenised duodenal mucosa, and the mean data expressed as μg CaBP per mg total supernatant protein.

calcaemic factor from *S. malacoxylon*, by a mixture of glycosidases, has been a prerequisite for receptor binding⁶. Thus we exposed (*in vitro*) an extract of *C.d.* to this same mixture of glycosidases from the liver of the seaworm, *Charonia lampas*, and obtained a lipid-soluble biologically active fraction. This hydrolysed material was then purified by a series of chromatographic columns (see legend of Fig. 1). The final step was Celite chromatography and the 1,25-(OH)₂D₃-like binding activity comigrated with authentic tritiated 1,25-(OH)₂D₃ (Fig. 1b), as also observed with the purified plasma principle from *C.d.*-fed strontium-inhibited chicks (Fig. 1a). It is thus reasonable to assume that the *in vitro* hydrolysis by the mixed glycosidase preparation resembles a similar hydrolysis which takes place *in vivo*, both liberating an active fragment indistinguishable from 1,25-(OH)₂D₃ by all of the chromatographic systems used (see Fig. 1 legend).

The chromatography scheme used analytically in these co-migration studies was then used to purify sufficient quantities of *C. lampas*-hydrolysed material to allow for further characterisation of the *C.d.* factor. The pooled peak fractions from the final Celite column exhibited an ultraviolet absorption spectrum identical to that of 1,25-(OH)₂D₃ (ref. 16). Quantitation of the material was achieved with the radioreceptor assay which indicated that 1.2 µg of 1,25-(OH)₂D₃-equivalents were present. This represents the total activity derived from 300 g of dried *C.d.* leaf, but this should not be used as an indication of the concentration of the factor in *C.d.*, since we were unable to assess yields during the early extraction procedures.

This evidence for the 1,25-(OH)₂D₃-like nature of the *C.d.* principle led us to compare the mass spectrum of the hydrolysed purified *C.d.* extract to that of crystalline 1,25-(OH)₂D₃ by gas chromatography/mass spectrometry (GC/MS). D-vitamins are known to pyrolyse during gas chromatography yielding two isomers—the 'pyro-' and 'isopyro-' forms, as a result of closure of the B-ring^{8,17}; this fact is germane to understanding the fragment pattern. Figure 2a (inset) depicts the total ion current obtained with 1.2 µg of synthetic 1,25-(OH)₂D₃. The major peak of ion current emerges from the column at 7 min (arrow) and represents 1,25-dihydroxypyrovitamin D₃; the second peak represents 1,25-dihydroxyisopyrovitamin D₃. The mass spectrum

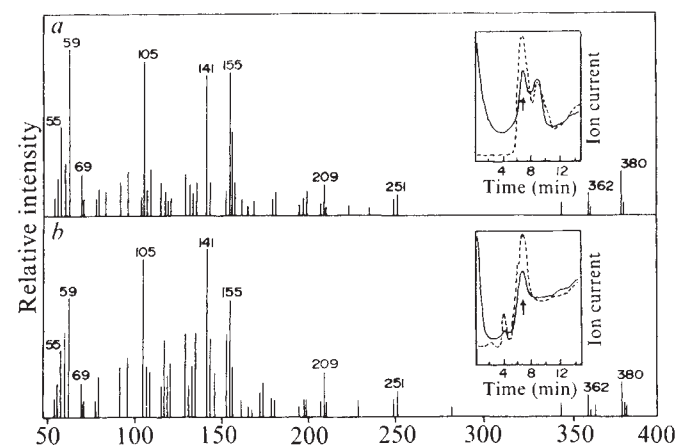


Fig. 2 Analysis of 1,25-(OH)₂D₃ and the lipophilic fragment of *C.d.* by gas-liquid partition chromatography and mass spectrometry. *a*, Gas chromatogram/mass spectrum of 1.2 µg of synthetic 1,25-(OH)₂D₃. *b*, Gas chromatogram/mass spectrum of 1.2 µg of purified hydrolysed *C.d.* Insets show gas chromatographic traces of total ion current (solid line) and ion current at *m/e* 380 (broken line). The arrows indicate the peak fractions which were characterised by mass spectrometry. These analyses were performed on a Model 3300-6110 Finnigan GC/MS with quadrupole analyser in the electron impact ionisation mode, equipped with a 2 mm × 50 cm, 3% OV-7 (100-120 mesh) glass column. Other parameters: injection port temperature, 260 °C; column temperature, 245 °C initial, 8 °C per min programmed increase; sample application in 5 µl double distilled ethanol (100%).

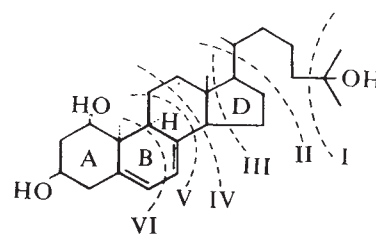


Fig. 3 Proposed fragmentation pattern for 1,25-dihydroxypyrovitamin D₃. The molecular weight of 1,25-(OH)₂D₃ is 416, but the parent peak of the GC/MS, which is at *m/e* 380, represents the native molecule minus two hydroxyl groups that are lost in the form of H₂O during heating. Loss of the third hydroxyl in the form of H₂O yields the fragment at *m/e* 362. Cleavage I gives a major peak at *m/e* 59 by removal of the portion of the side chain; and, removal of the entire sidechain (111 AMU) from the fragment at *m/e* 362 (cleavage II) yields the peak at *m/e* 251 which is the fused ABCD ring array. As discussed by Budzikiewicz and coworkers¹⁹, steroid ring systems are commonly cleaved between C₁₄-C₁₅ and C₁₃-C₁₇ (cleavage III) losing 42 AMU and leaving a fragment at *m/e* 209 in this instance. The *m/e* 209 fragment (or *m/e* 210 resulting from common hydrogen rearrangement in steroids¹⁹), now serves as a source for three complementary pairs of fragments which comprise the important remaining peaks of the 1,25-(OH)₂D₃ mass spectral pattern. These pairs include: *m/e* 155 and 55 resulting from cleavage at IV; *m/e* 141 and 69, from cleavage at V; and *m/e* 105 which represents both fragments generated from cleavage at VI. It should be emphasised that cleavages III-VI are characteristic cleavage positions predicted from the mass spectra of other steroids^{18,19}.

(Fig. 2a) of the major peak (arrow) shows characteristic fragments at *m/e* 380, 362, 251, 209, 155, 141, 105, 69, 59, and 55. A similar mass fragment pattern was observed for the second, 'isopyro', peak of total ion current (data not shown). Figure 3 shows 1,25-dihydroxypyrovitamin D₃ and the cleavages of the ABCD ring system which are typical of classic steroids¹⁸ and probably produce the mass fragments actually observed (Fig. 2a) for 1,25-(OH)₂D₃.

The inset of Fig. 2b illustrates the total ion current trace and the monitored ion current at *m/e* 380 obtained from gas chromatography of 1.2 µg of purified hydrolysed *C.d.* factor. Again, the major peak (arrow) emerges at 7 min, resolved from sample contaminants which apparently obscure any observation of the 'isopyro-' form of *C.d.* Mass spectral analysis of this peak (Fig. 2b) reveals a fragment pattern equivalent to that obtained for 1,25-(OH)₂D₃. The coincident migration of the active lipophilic fragment of *C.d.* with 1,25-(OH)₂D₃ on the gas chromatographic column, and the identical mass spectra of the resulting peaks convincingly demonstrates that 1,25-(OH)₂D₃ is present in *C.d.*, although the steroid apparently exists in the native plant in the form of a glycoside.

This work was supported by NIH grants AM-04632, AM-15355, AM-15781, and training grant GM-01982.

MARK R. HUGHES
TONI A. MCCAIN
SAI Y. CHANG
MARK R. HAUSSLER

Departments of Biochemistry
and Internal Medicine,
College of Medicine,
University of Arizona,
Tucson, Arizona 85724

MICHAEL VILLAREALE
ROBERT H. WASSERMAN

Department of Physical Biology,
New York State College of Veterinary Medicine,
Cornell University,
Ithaca, New York 14853

Received 21 March; accepted 31 May 1977.

1. Krook, L. P. *et al.* *Cornell Vet.* **65**, 26-56 (1975).
2. Krook, L. P., Wasserman, R. H., McEntree, K., Brokken, T. D. & Toigland, M. B. *Cornell Vet.* **68**, 537-578 (1975).

- 3 Wasserman, R. H. *Nutr. Rev.* 33, 1-5 (1975).
- 4 Wasserman, R. H. & Corradino, R. A. *Biochem. biophys. Res. Commun.* 62, 85-91 (1975).
- 5 Wasserman, R. H., Corradino, R. A., Krook, L. P., Hughes, M. R. & Haussler, M. R. *J. Nutr.* 106, 457-465 (1976).
- 6 Peterlik, M., Bursac, K., Haussler, M. R., Hughes, M. R. & Wasserman, R. H. *Biochem. biophys. Res. Commun.* 70, 797-804 (1976).
- 7 Haussler, M. R. *et al. Life Sci.* 18, 1049-1056 (1976).
- 8 Wasserman, R. H., Henion, J. D., Haussler, M. R. & McCain, T. A. *Science* 194, 853-855 (1976).
- 9 Wasserman, R. H. *Science* 183, 1092-1094 (1974).
- 10 Brumbaugh, P. F., Haussler, D. H., Bressler, R. & Haussler, M. R. *Science* 183, 1089-1091 (1974).
- 11 Haussler, M. R. *et al. Clin. Endocrin.* 5, 151s-165s (1976).
- 12 Hughes, M. R., Baylink, D. J., Jones, P. G. & Haussler, M. R. *J. clin. Invest.* 58, 61-70 (1976).
- 13 Corradino, R. A. *J. Cell Biol.* 58, 64-78 (1973).
- 14 Hughes, M. R., Baylink, D. J., Gonnerman, W. A., Toverud, S. U., Ramp, W. K. & Haussler, M. R. *Endocrinology* 100, 187-194 (1977).
- 15 Spencer, R., Charman, M., Wilson, P. & Lawson, E. *Nature* 263, 161-163, (1976).
- 16 Holick, M. F., Schnoes, H. K., DeLuca, H. F., Suda, T. & Cousins, R. J. *Biochemistry* 10, 2799-2804 (1971).
- 17 Ziffer, H., Vanden-Heuvel, W. J. A., Haathi, E. O. A. & Horning, E. C. *J. Am. Chem. Soc.* 82, 6411-6412 (1960).
- 18 Waller, G. R. *Biochemical Applications of Mass Spectroscopy* (Wiley-Interscience, New York, 1972).
- 19 Budzikiewicz, H., Djerassi, C. & Williams, D. H. *Structure Elucidation of Natural Products by Mass Spectroscopy* (Holden-Day, San Francisco, 1964).

Synthesis, storage and release of acetylcholine by a noradrenergic pheochromocytoma cell line

DEVELOPING neural crest tissues show a certain degree of plasticity with respect to neurotransmitter synthesis. For example, heterotopic transplants of presumptive noradrenergic tissue may develop cholinergic properties and vice versa^{1,2}. Also, monolayer cultures of rat sympathetic neurones which synthesise catecholamines may be influenced to synthesise acetylcholine by exposure to ganglionic non-neuronal cells or to medium conditioned by such cells^{3,4}. Recently, a clonal cell line (designated PC12) was established⁵ from a transplantable rat adrenal medullary pheochromocytoma⁶. This line expresses many characteristic properties of neural-crest-derived noradrenergic adrenal chromaffin cells and sympathetic neurones, including synthesis, storage, uptake and specific release of catecholamines^{5,7,8}. In addition, within several days after their exposure to nerve growth factor (or NGF, a protein which influences the growth and development of normal sympathetic neurones⁹), PC12 cells cease mitotic activity and extend long, neuronal processes⁵. We show here that noradrenergic cultures of PC12 cells can also synthesise, store and release acetylcholine.

PC12 cultures, maintained as described before^{5,7} were assayed for choline acetyltransferase (CAT) activity by the method of Fonnum¹⁰. Assays performed in the absence of choline or in the presence of 100 μ M naphthylvinyl pyridine (a specific inhibitor of CAT), gave values similar to tissue-free blanks, thus indicating the specificity of the activity for CAT and excluding the presence of carnitine acetyltransferase¹¹. As shown in Fig. 1a, b, the specific activity (s.a.) of CAT varied markedly with cell density. At maximal cell density, specific activities reached 500-600 pmol per min per mg protein. This level is comparable with magnitude to that observed in rat brain tissue¹² but is lower than levels reported in chicken ciliary ganglia¹³ (~50 nmol min⁻¹ mg⁻¹). The increase in the s.a. of CAT in PC12 cultures occurred mainly during the logarithmic stage of cell growth and then plateaued when the cultures reached the stationary phase (Fig. 1a, b). Thus, the s.a. of CAT is apparently related to cell density rather than to the rate of cell division. As further evidence of this, CAT activities in cultures of division-arrested PC12 cells (produced by treatment with 1 μ M cytosine arabinoside) were comparable to those in logarithmic growing cultures of similar density (see zero time in Fig. 1c, for example). Such findings suggest that the increase in specific CAT activity with increased cell density may be due to auto-conditioning in a manner analogous to that observed for

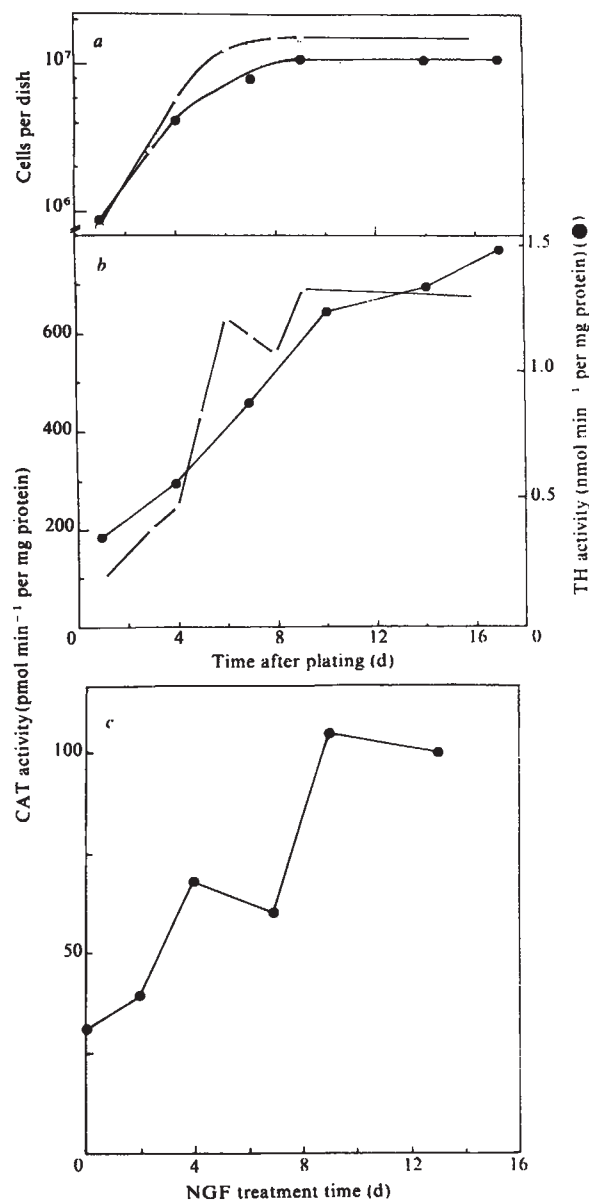


Fig. 1 Regulation of CAT activity in PC12 cells. *a, b*, Variation of specific activities of CAT (○) and TH (●) as a function of cell density. *a*, Cell number; *b*, specific enzyme activities. Cells were plated in collagen-coated 35-mm dishes as previously described⁵. In this, and in all subsequent experiments, the culture medium was changed every other day. At indicated times, cells were collected from duplicate sister cultures and assayed in duplicate for cell number and enzyme activity. CAT activity was measured according to Fonnum¹⁰; TH was measured according to Black *et al.*¹⁹, with concentrations of tyrosine and DMPH₄ of 200 μ M and 1 mM, respectively. Specific activities are based on protein levels of homogenates estimated (with a bovine serum albumin standard) by the methods of Lowry *et al.*²⁰. (TH assays) or McGuire *et al.*²¹ (CAT assays). Variation in specific activities between duplicate cultures in this and other experiments was less than 15%. *c*, Effect of NGF treatment on the specific activity of CAT in division-arrested PC12 cells. Non-dividing PC12 cells were produced as follows. Stationary phase dishes (100 mm) of PC12 cells were exposed to 1 μ M cytosine arabinoside (ara-C) for one week. The cells which survived this treatment (about 20% of the original population) were found to be non-dividing and to survive in the presence of ara-C for at least 1 month. The ara-C-selected cells were then subcultured into 35 mm collagen-coated dishes at low cell density (1.2×10^5 per dish) in the presence of ara-C. At various times after plating, NGF was added to the medium of pairs of sister cultures. On day 13, cells were collected from all cultures and assayed in duplicate for CAT. Cell counts confirmed that the cell number in both NGF-treated and untreated cultures did not change significantly during the course of the experiment.

induction of CAT in cultures of sympathetic neurones by non-neuronal cells³.

The presence of CAT activity in PC12 cells was not only limited to tissue cultures; tumours of PC12 cells grown subcutaneously in rats had CAT activities on the order of $200 \text{ pmol min}^{-1} \text{ mg}^{-1}$. Furthermore, we have detected CAT activity in tissue from two catecholamine-secreting human pheochromocytomas (unpublished).

Parallel experiments on PC12 cultures revealed that the s.a. of tyrosine hydroxylase (TH), the first enzyme in the synthetic pathway of catecholamines, also increased as a function of cell density (Fig. 1a, b). Thus, both CAT and TH seem to be similarly inducible in PC12 cultures and to be at maximal levels at high cell density. Such findings lead to the question of whether both neurotransmitter synthesising systems coexist in the same cells. Several pieces of evidence suggest (but do not unequivocally prove) that this is so. First, as judged by histochemical localisation, virtually every PC12 cell in high density cultures contains catecholamines^{3,14}. Secondly, ultrastructural studies of PC12 cultures have revealed that dense-core granules (of the type thought to contain catecholamines) and small agranular vesicles (of the type usually considered to contain acetylcholine) are present in the same cells^{3,14}. To test further the possibility of dual neurotransmitter synthesis, PC12 cells (250 generations after isolation) were subcloned as previously described⁵. Table 1 reveals that of the single-cell subclones isolated and tested at high cell density, all seven had high levels of TH activity while two also displayed reasonably high levels of CAT activity. This suggests that although the PC12 line is heterogeneous with respect to CAT activity, at least a given subpopulation of cells contains both enzymes. Further experiments will be needed to determine whether such subclones will themselves remain homogenous for CAT activity and whether CAT activity may be induced (either spontaneously or by treatment with conditioned media) in the subclones with low levels of this enzyme.

As mentioned above, PC12 cells respond to NGF by cessation of replication and extension of neurites. To access the effect of NGF treatment on CAT activity in PC12 cells and to dissociate such effects from those on cell division (and thus cell density as well), the following experiment was performed. A stable population of division-arrested PC12 cells was produced by treatment with $1 \mu\text{M}$ cytosine arabinoside (ara-C) for one week. Such cells were then subcultured at low density (1.2×10^5 per 35-mm dish) in the presence of ara-C, treated with 2.5 S NGF (ref. 15) for various periods and then harvested and assayed for CAT activity. The ara-C treated cells began to extend neurites within 2 d of treatment with NGF, but did not do so in its absence. The results of

Table 2 Synthesis and storage of ACh by PC12 cultures

	Untreated	NGF-treated
CAT activity		
pmol per min per mg protein	440 ± 17	520 ± 20
pmol per min per 10^6 cells	31 ± 2	83 ± 5
ACh content		
pmol per mg protein	$1,335 \pm 77$	$5,594 \pm 637$
pmol per 10^6 cells	93 ± 7	892 ± 146

Data are mean values ($\pm$ s.e.m.) based on assays performed in quadruplicate on NGF-treated (22 d in the presence of 50 ng ml^{-1} NGF, 3.5×10^6 cells per dish) and high density untreated (13.6×10^6 cells per dish) PC12 cultures.

this experiment (Fig. 1c) revealed that the s.a. of CAT underwent a significant increase within several days of NGF treatment. Furthermore, the time course of this increase roughly paralleled that of neurite outgrowth. No conclusions regarding a causal relationship between the two events could be drawn, however.

Experiments were also carried out to assess whether PC12 cells could store the acetylcholine (ACh) they synthesised and whether NGF treatment would affect this capacity. Since PC12 cells (NGF-treated and untreated) contain high levels of cytoplasmic acetylcholinesterase activity (F. Rieger, P. Taylor and L.A.G., in preparation), it is likely that any endogenous ACh is stored rather than free in the cytoplasm. Accordingly, cultures of NGF-treated and of high-density NGF-untreated PC12 cells with comparable specific activities of CAT were assayed for their contents of ACh by the sensitive radioassay of Goldberg and McCaman¹⁶ (Table 2). Although both types of cultures contained substantial quantities of ACh (nmol per mg protein), those treated with NGF contained three- to fourfold more ACh per mg protein and an average of about tenfold more per cell. One possible basis for this difference in content is that NGF-treated PC12 cells contain many more small agranular vesicles than do untreated cells^{3,14}.

The data in Table 3 show the results of experiments which demonstrate that PC12 cells can not only synthesise and store ACh, but also release it on exposure to a depolarising medium. While release of ^3H -ACh was significantly enhanced in the presence of 51.5 mM K^+ , release of ^3H -choline was not. In three separate experiments, the proportion of the cellular contents of labelled ACh released by elevated K^+ was between 15 and 20%. We have previously obtained comparable results for the release of noradrenaline from PC12 cells in similar conditions⁷. Thus, it is possible that both neurotransmitters may be secreted by the same cells. Evidence for such a dual release has been presented for rat sympathetic neurons in culture¹⁷. Finally, as shown for nerve endings and synaptosomes (ref. 18) the K^+ -induced increase in release of ACh from PC12 cells was blocked in media either free of Ca^{2+} or containing elevated ($10 \times$ control) Mg^{2+} .

In conclusion, cultures of the noradrenergic PC12 line of rat pheochromocytoma may synthesise, store and release significant quantities of ACh. The duality of neurotransmitter function shown by this type of cell may resemble, as well as have common origins with, that shown by other neural crest derivatives of normal and neoplastic origin. In this context, PC12 cells should prove useful in the study of various aspects of acetylcholine metabolism as well as the regulation and specification of neurotransmitter properties in developing neurones.

We thank Mrs M.-N. Chou for assistance with tissue culture and Drs P. Patterson, S. R. Snodgrass, H. Thoenen and N. Uretsky for helpful discussion. This work was

Table 1 Enzymatic activities of subclones of PC12 cells

Subclone	Specific enzymatic activity (pmol per min per mg protein)	
	TH	CAT
1A	2,866	11
2B	3,468	170
3A	2,315	15
3B	1,847	8
D2	2,522	153
B1	3,054	19
C2	2,193	14

Single cell subclones were isolated as previously described⁵. Clones were grown to high density (20 generations after initial isolation), harvested and assayed for specific enzymatic activity. Results are means of duplicate assays on first (TH) or first and second (CAT) passage cultures. Lower limit of sensitivity of CAT assay was $0.5 \text{ pmol per min per mg protein}$.

Table 3 Release of ACh from PC12 cells

Release conditions	d.p.m. released per culture of Choline	ACh
Control	27,750 ± 5,550	6,110 ± 2,830
51.5 mM K ⁺	33,860 ± 2,890	21,650 ± 3,330
51.5 mM K ⁺ , 0 Ca ²⁺	28,310 ± 2,220	4,440 ± 2,940
51.1 mM K ⁺ , 10 × Mg ²⁺	30,530 ± 2,810	11,100 ± 2,330
Intracellular content before release	71,040 ± 6,660	108,225 ± 8,800

Sister cultures (collagen-coated 35-mm dishes containing 2×10^6 cells) of NGF-treated (14 d) PC12 cells were washed free of culture medium and incubated (at 37 °C in this and all subsequent incubations) for 30 min in 1 ml of HEPES-buffered Ringers saline (KRH) (ref. 7) supplemented with (methyl-³H)-choline (10.7 µCi per dish; s.a. = 2.5 Ci mmol⁻¹). The 'loading' medium was then removed and the cultures were washed three times with saline and then maintained for 1 h with 1.5 ml of culture medium RPMI 1640 supplemented with 2% horse serum to allow washout of unbound ³H-choline and its derivatives. Cultures were then pre-incubated for 15 min in KRH before successive 20-min incubations with 1 ml aliquots of KRH (control medium lacking Ca²⁺ or with 10 × Mg²⁺ also gave comparable final values) and then KRH modified as described above to contain 51.5 mM K⁺ and the indicated levels of Ca²⁺ and Mg²⁺. Eserine (30 µM) was also present during incubations to inhibit cellular acetylcholine esterase. The levels of ³H-choline and ³H-acetylcholine released by and contained within the cells were determined as follows by modifications of previously described procedures^{10,16,18,22}. The KRH fractions were placed on ice immediately after the incubations and shortly thereafter extracted with 3 ml of heptan-3-one containing 20 mg ml⁻¹ tetraphenylboron. Choline and ACh were then extracted from the organic phase in 1 ml of 0.4 M HCl which was then lyophilised to dryness. Choline and ACh remaining within the cells after release was collected by homogenising in 500 µl of 1 M formic acid-acetone (15:85 v/v). The homogenate was centrifuged at 1000g for 5 min and the supernatant was taken to dryness *in vacuo*, resuspended in 1 ml of KRH and then extracted and lyophilised as above. Each dry sample was re-dissolved in 40 µl of chromatography solvent containing authentic carrier choline and ACh (each 10 mM) and 25 µl of this was spotted onto thin layer chromatography (TLC) plates (Eastman no. 6064). After chromatography as previously described²² the plates were dried and the locations of carrier ACh and choline were identified by exposure to I₂ vapour. Each chromatography track was then cut into 5-mm horizontal strips which were placed in separate glass vials and extracted with 1 ml of 0.1 M HCl for 30 min. Liquid scintillation fluid (Instagel-Packard) was added to the vials which were then assayed for radioactivity at a counting efficiency of 23%. Data are presented as radioactivity which co-chromatographed with authentic ACh and choline. Recoveries were estimated on the basis of that of samples of ³H-choline which were similarly processed (ACh has been shown previously to have a comparable recovery to that of choline¹⁸). Values are given (±s.d.) for an experiment with seven cultures (three for 51.5 mM K⁺ and two each for 0 Ca²⁺ and 10 × Mg²⁺). Comparable release data were obtained in two other separate experiments.

supported in part by grants from the USPHS, National Foundation-March of Dimes and the Sloan Foundation.

LLOYD A. GREENE
GLEN REIN

Department of Neuropathology, Harvard Medical School
and Department of Neuroscience,
Children's Hospital Medical Center,
300 Longwood Avenue, Boston, Massachusetts 02115

Received 7 April; accepted 6 June 1977.

- LeDourarin, N. M., Renaud, D., Teillet, M. A. & LeDourarin, G. H. *Proc. natn. Acad. Sci. U.S.A.* **72**, 728-732 (1975).
- LeDourarin, N. M. & Teillet, M. A. M., *Dev. Biol.* **41**, 162-184 (1974).
- Patterson, P. H. & Chun, L. L. Y. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3607-3610 (1974).
- Patterson, P. H., Reichardt, L. F. & Chun, L. L. Y. *Cold Spring Harb. Symp. quant. Biol.* **40**, 389-397 (1976).
- Greene, L. A. & Tischler, A. S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2424-2428 (1976).
- Warren, S. & Chute, R. *Cancer Res.* **32**, 327-331 (1972).
- Greene, L. A. & Rein, G. *Brain Res.* **129**, 247-263 (1977).
- Greene, L. A. & Rein, G. *Brain Res.* (in the press).
- Levi-Montalcini, R. & Angeletti, P. U. *Physiol. Rev.* **48**, 534-569 (1968).
- Fonnum, F. *Biochem. J.* **115**, 465-472 (1969).
- White, H. L. & Wu, J. C. *Biochemistry* **12**, 841-846 (1973).

- Cheney, D. L., Racagni, G. & Costa, E. in *Biology of Cholinergic Function* (eds Goldberg, A. M. & Hanin, I.) 655-659 (Raven, New York, 1976).
- Sorimachi, M. & Kataoka, K. *Brain Res.* **70**, 123-130 (1974).
- Tischler, A. S. & Greene, L. A. *In vitro* **13**, 156 (1977).
- Bocchini, V. & Angeletti, P. U. *Proc. natn. Acad. Sci. U.S.A.* **64**, 787-794 (1969).
- Goldberg, A. M. & McCaman, R. E. *J. Neurochem.* **20**, 1-8 (1973).
- Furshpan, E. J., MacLeish, P. R., O'Lague, P. H. & Potter, D. D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4225-4229 (1976).
- Wonnacott, S. & Marchbanks, R. M. *Biochem. J.* **156**, 701-712 (1976).
- Black, I. B., Hendry, I. A. & Iversen, L. L. *Brain Res.* **34**, 229-240 (1971).
- Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265-275 (1951).
- McGuire, J., Taylor, P. & Greene, L. A. *Analyt. Biochem.* (in the press).
- Marchbanks, R. M. & Israël, M. *J. Neurochem.* **18**, 439-448 (1971).

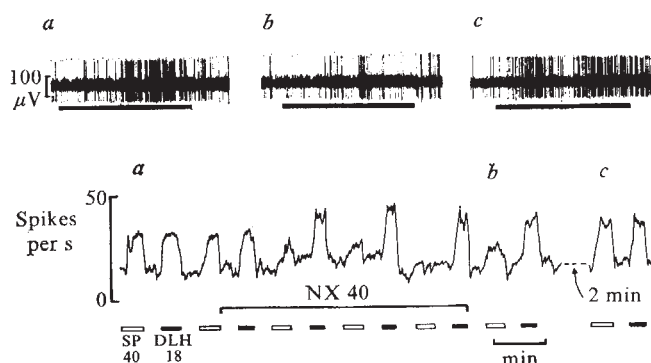
Substance P and opiate receptors

It has recently been demonstrated that the undecapeptide substance P (SP) produces analgesia in mice when administered intracerebroventricularly (i.v.) or intraperitoneally (i.p.).¹ This effect of SP was reversed by naloxone indicating the possible involvement of central opiate receptors. The possibility that SP may act on opiate receptors at the single neurone level has been investigated on spinal Renshaw cells in the cat. These cells possess stereo-specific opiate receptor sites^{2,3}. The results obtained show that SP excites Renshaw cells and that this action is antagonised by naloxone. Substance P also reduced the excitation of Renshaw cells by acetylcholine.

Experiments were performed in 12 cats anaesthetised with pentobarbitone sodium (35 mg kg⁻¹ i.p. initially and supplemented i.v. as required). Blood pressure was continuously monitored and body temperature was maintained at 37 °C. A lumbar laminectomy was performed and the cut central ends of S1 or L7 ventral roots were stimulated to identify Renshaw cells. Extracellular action potentials were recorded *via* the centre barrel (4M NaCl) of a seven barrel micropipette (tip diam. 6 µm). The outer barrels of the micropipette contained aqueous solutions of the following drugs to be ejected electrophoretically: substance P (0.0007 M in 0.165 M NaCl), morphine sulphate (0.05 M in 0.165 M NaCl), naloxone hydrochloride (0.1 M), acetylcholine chloride (0.5 M), DL-homocysteic acid (0.2 M, pH 7.2), NaCl (0.165 M).

Substance P excited 17 of 34 Renshaw cells when ejected with cationic currents (40-80 nA, mean 58 nA). Excitation by SP was invariably marked, of rapid onset and comparable to that of DL-homocysteic (see Fig. 1). On, five cells however, the excitatory effects of SP were of slower onset and outlasted the duration of electrophoretic ejection by 5-20 s. The differences in the time course of SP excitation in the present experiments and the inconsistent effects of SP in other studies⁵ may be associated

Fig. 1 Rate meter record (spikes per s against min) illustrating excitation of a Renshaw cell by alternate iontophoretic ejection of substance P (SP 40 nA) and DL-homocysteic (DLH, 18 nA). Ejection of naloxone (NX 40 nA) from an adjacent barrel of the same pipette reversibly reduced SP but not DLH-induced excitation. Extracellular spike records *a*, *b* and *c* of substance P effects correspond to the labelled rate meter response. The duration of iontophoretic ejections is indicated by the horizontal bars below each record. The spike records also show that SP excitations were not accompanied by changes in spike amplitude.



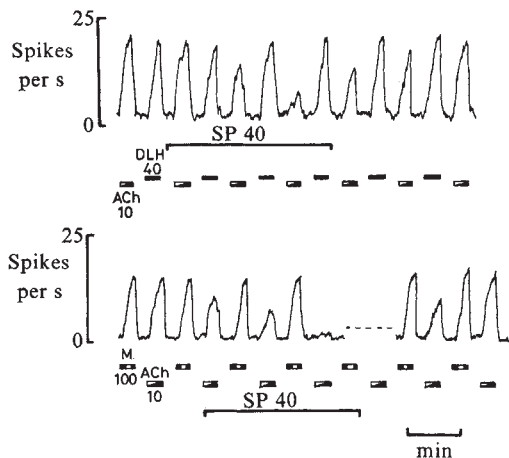


Fig. 2 Rate meter record showing excitation by alternate ejections of acetylcholine (ACh 10 nA) and DLH (40 nA). Prolonged electrophoretic administration of substance P had no effect on the spontaneous firing of this cell but SP (40 nA) reversibly reduced ACh and not DLH responses. The lower record from another Renshaw cell shows that a similar prolonged ejection of SP again reversibly reduced ACh excitation but had little effect, at this current (40 nA), on morphine (100 nA)-induced excitation. Recovery of ACh sensitivity was more prolonged—the dashed line indicates a break of 3 min. A higher current of SP also reduced excitation by morphine on this cell (not shown).

with variable rates of release from individual micropipettes^{4,5}. No desensitisation or tachyphylaxis of SP-induced excitation was observed though this phenomena may be a feature accompanying SP excitation in other areas of the CNS (refs 4–8).

Substance P-induced excitation was reversibly reduced by the narcotic antagonist naloxone (30–40 nA, mean 36 nA) on five of the six neurones, whereas the excitatory action of DL-homocysteic acid was unaffected on three cells and slightly enhanced on the others (Fig. 1). On the same cells, the excitatory effects of morphine and acetylcholine were also reversibly reduced by naloxone in agreement with earlier findings^{2,3,9}.

In contrast, prolonged ejections of SP with relatively low currents (20–40 nA, mean 30 nA for 2 min or more) markedly reduced the sensitivity of Renshaw cells to acetylcholine while having little effect on spontaneous firing or DL-homocysteic acid-induced excitation of the same cells (Fig. 2, upper record). This depression of acetylcholine sensitivity was often prolonged (Fig. 2, lower record). Similar results were obtained from 14 other Renshaw cells. This interaction of SP has been noted in another laboratory¹⁰ and we have previously described a similar reduction of acetylcholine sensitivity of Renshaw cells by another peptide, enkephalin¹¹.

In tests on three cells, SP antagonised both morphine and acetylcholine excitation. However, in each cell the acetylcholine responses were reduced by lower ejecting currents of SP (see Fig. 2). Interestingly, enkephalin also differentiates between excitation induced by morphine and acetylcholine¹¹.

The present results show that the excitatory effects of SP on Renshaw cells are remarkably similar to the excitatory effects of enkephalin and to the stereospecific effects of morphine^{2,3,11}. Additionally, the responses to these three substances are antagonised by naloxone. Thus, these excitatory effects of SP are consistent with an action on opiate receptors. Whether the activation of such receptors on the Renshaw cell is related to the analgesic actions of SP¹ or opiates (see refs 3, 9) is uncertain. However, it may be significant that the regional distribution of SP concentration and opiate receptor binding sites in other brain areas is similar^{12,13}. Thus, activation of opiate receptors in these areas might explain the analgesic actions of SP more readily.

The reduction of acetylcholine sensitivity produced by SP might be unrelated to an action at opiate receptors since morphine enhances acetylcholine responses on Renshaw cells^{3,9}.

However, this action of SP has been demonstrated in several brain areas^{5,8} and is shared by other peptides, for example, enkephalin¹¹ which suggests that polypeptides should be considered to exert a multiplicity of actions at central neurones.

This work was supported by an MRC programme grant to Professor D. W. Straughan. We are grateful to Endo Laboratories for gifts of naloxone hydrochloride.

J. DAVIES
A. DRAY

Department of Pharmacology,
The School of Pharmacy,
29/39 Brunswick Square,
London WC1, UK

Received 28 March; accepted 27 May 1977.

- ¹ Stewart, J. M., Getto, C. J., Neldner, K., Reeve, E. B., Krivoy, W. A. & Zimmerman, E. *Nature* **262**, 784–785 (1976).
- ² Davies, J. & Duggan, A. W. *Nature* **250**, 70–71 (1974).
- ³ Davies, J. *Brain Res.* **113**, 311–326 (1976).
- ⁴ Krnjevic, K. & Morris, M. E. *Can. J. physiol. Pharmacol.* **52**, 736–744 (1974).
- ⁵ Henry, J. L., Krnjevic, K. & Morris, M. E. *Can. J. physiol. Pharmacol.* **53**, 423–432 (1975).
- ⁶ Phillis, J. W. & Limacher, J. J. *Brain Res.* **69**, 158–163 (1974).
- ⁷ Henry, J. L. *Brain Res.* **114**, 439–451 (1976).
- ⁸ Davies, J. & Dray, A. *Brain Res.* **107**, 623–627 (1976).
- ⁹ Duggan, A. W., Davies, J. & Hall, J. G. *J. Pharmac. exp. Ther.* **196**, 107–120 (1976).
- ¹⁰ Belcher, G., Davies, J. & Ryall, R. W. *J. Physiol., Lond.* (in the press).
- ¹¹ Davies, J. & Dray, A. *Nature* **262**, 603–604 (1976).
- ¹² Kanazawa, I. & Jessell, T. *Brain Res.* **117**, 362–367 (1976).
- ¹³ Snyder, S. H. *Nature* **257**, 185–189 (1975).

Evidence for a cyclic nucleotide-mediated calcium flux in motor nerve terminals

WE recently suggested that a cyclic nucleotide system might participate in neuromuscular transmission by regulating the flux of calcium and/or sodium into the nerve ending^{1–3}. The purpose of the present investigation was to refine this postulate by studying the interactions between the cyclic nucleotide system and reagents that affect calcium and sodium flux in nerve terminals differentially. Recording from single motor axons of cat soleus nerves *in vivo*, we found that the calcium antagonists verapamil (ipoveratril)^{4,5} and lanthanum⁶ blocked stimulus-bound repetitive action potentials (SBR) produced by the administration of dibutyryl (db) cyclic AMP and sodium fluoride, whereas tetrodotoxin (TTX) did not alter the nerve or muscle response to these reagents. The results suggest that an intraterminal cyclic nucleotide system may regulate calcium flux into the motor nerve ending.

The preparation has been described before⁷. Cats were anaesthetised with 70 mg kg⁻¹ α -chloralose and the popliteal fossa was exposed. All branches of the popliteal artery except that which supplies the soleus muscle were occluded and all branches of the tibial nerve except that to the soleus muscle were severed. Both heads of the gastrocnemius muscle were removed and the tendon of the soleus muscle was attached to a strain gauge. A dorsal laminectomy was performed, the ventral root of L7 was cut and its distal stump was split until a strand containing a single functional axon to the soleus muscle was found. This strand was placed over bipolar platinum recording electrodes. A bipolar platinum stimulating electrode was placed on the soleus nerve near its entry into the muscle and supramaximal pulses lasting 0.1 ms were applied every 2.5 s. When appropriate, tetanic stimulations of 400 Hz for 10 s were applied every 5 min. The following materials were injected into the popliteal artery: N⁶-2'-O-dibutyryl-adenosine-3', 5'-monophosphoric acid, cyclic (db cyclic AMP), sodium fluoride (NaF), lanthanum chloride (LaCl₃), tetrodotoxin (TTX) and verapamil (a gift from Knoll laboratories). Each com-

pound was dissolved in 0.85% NaCl solution so that 0.1 ml contained the amount to be administered per kg.

Forty injections of verapamil ($0.001\text{--}200\text{ }\mu\text{g kg}^{-1}$) were administered to seven animals. Doses lower than $0.01\text{ }\mu\text{g kg}^{-1}$ had no effect on the SBR induced by db cyclic AMP but $0.1\text{ }\mu\text{g kg}^{-1}$ depressed it in all seven preparations (Fig. 1). It completely prevented the production of SBR in five of these and reduced the duration of repetitive activity and the total number of repetitive potentials in the others ($8\pm 15\%$ (s.e.) and $6\pm 3\%$ of control, respectively). The administration of higher doses (up to $200\text{ }\mu\text{g kg}^{-1}$) did not increase the effect of depression; it was impossible to prevent SBR completely in all fibres. Verapamil ($0.1\text{ }\mu\text{g kg}^{-1}$) also reduced the potentiation of muscle force caused by the transmission of the neural SBR to the muscle ($20\pm 4\%$ of control) (Fig. 1). In addition, verapamil reduced the brief bursts of action potentials in the motor axons and the rapid asynchronous action potentials and contractions in the muscle which were induced by the administration of db cyclic AMP to preparations in which the stimulator was turned off (drug-

induced activity, DIA). Recovery from verapamil occurred within 5–15 min.

The effects of verapamil on the responses to NaF ($100\text{ }\mu\text{g kg}^{-1}$) were the same as on those to db cyclic AMP. Verapamil ($0.1\text{ }\mu\text{g kg}^{-1}$) blocked SBR in six of eight preparations and significantly reduced the duration of repetitive activity and the total number of repetitive potentials in the others ($15\pm 10\%$ and $17\pm 11\%$ of control, respectively). Verapamil also reduced the potentiation by NaF of indirectly evoked muscle contractions ($15\pm 6\%$ of control) and diminished the DIA produced by the administration of NaF to unstimulated preparations.

Thirty-five injections of LaCl_3 ($0.01\text{--}100\text{ }\mu\text{g kg}^{-1}$) were administered to six animals. A dose of $0.01\text{ }\mu\text{g kg}^{-1}$ significantly prevented SBR produced by the administration of db cyclic AMP ($200\text{ }\mu\text{g kg}^{-1}$). It completely prevented the production of SBR in four of these and reduced the duration of repetitive activity and the total number of repetitive potentials in the others ($17\pm 9\%$ and $15\pm 8\%$ of control, respectively). Administration of higher doses (up to $100\text{ }\mu\text{g kg}^{-1}$) did not increase the degree of suppression; it was impossible to prevent SBR in all preparations. LaCl_3 ($0.01\text{ }\mu\text{g kg}^{-1}$) also significantly reduced the potentiation of muscle contraction produced by the administration of db cyclic AMP to indirectly-stimulated preparations and diminished DIA. Recovery from LaCl_3 occurred within 5–15 min.

The effects of LaCl_3 on the responses to NaF ($100\text{ }\mu\text{g kg}^{-1}$) were also the same as on those to db cyclic AMP. LaCl_3 ($0.01\text{ }\mu\text{g kg}^{-1}$) blocked SBR in five of seven preparations (Fig. 2) and significantly reduced the duration of SBR and the total number of repetitive potentials in the others ($20\pm 12\%$ and $21\pm 12\%$ of control, respectively). LaCl_3 also reduced the potentiation of indirectly evoked muscle contractions induced by NaF ($37\pm 6\%$ of control) (Fig. 2) and diminished DIA.

Tetrodotoxin ($0.01\text{ }\mu\text{g kg}^{-1}$) was administered to five animals. This dose significantly depressed the repetitive neural activity which follows high frequency stimulation (post-tetanic repetition, PTR); the duration of PTR and the total number of post-tetanic repetitive potentials were reduced to $48\pm 6\%$ and $45\pm 6\%$ of control, respectively and also reduced the potentiation of muscle force caused by the transmission of the neural PTR to the muscle ($64\pm 4\%$ of control) (Fig. 3). In some cases, this dose of TTX also impaired neuromuscular transmission. Since the generation of PTR is thought to be related to sodium accumulation in the nerve during high frequency stimulation, its reduction by TTX was expected^{8–10}. At $0.01\text{ }\mu\text{g kg}^{-1}$, however, TTX did not significantly alter either the duration of SBR or the total number of repetitive potentials produced by the administration of db cyclic AMP ($200\text{ }\mu\text{g kg}^{-1}$) ($121\pm 14\%$ and $103\pm 12\%$ of control, respectively) (Fig. 3). Similarly, the SBR produced by NaF ($100\text{ }\mu\text{g kg}^{-1}$) was unaffected by the administration of TTX; neither the duration of SBR ($97\pm 13\%$ of control) nor the number of repetitive potentials ($96\pm 7\%$ of control) was changed.

Stimulus-bound repetitive activity is thought to occur when the negative after-potential of the nerve terminal is prolonged for a period longer than the refractory period of the proximal portion of the axon. In this condition, the nerve terminal can act as a current sink for the proximal portion of the axon and re-excite it, thus giving rise to repetitive activity^{9,10}. These considerations previously led us to suggest that db cyclic AMP and NaF prolong the after-negativity of action potentials in motor nerve terminals^{1–3}.

Since cyclic nucleotides have been shown to facilitate the influx of both sodium and calcium into cells^{11–14}, either ion could account for the SBR observed after the administration of db cyclic AMP or NaF. The finding that doses of TTX adequate to reduce the sodium-related PTR did not alter

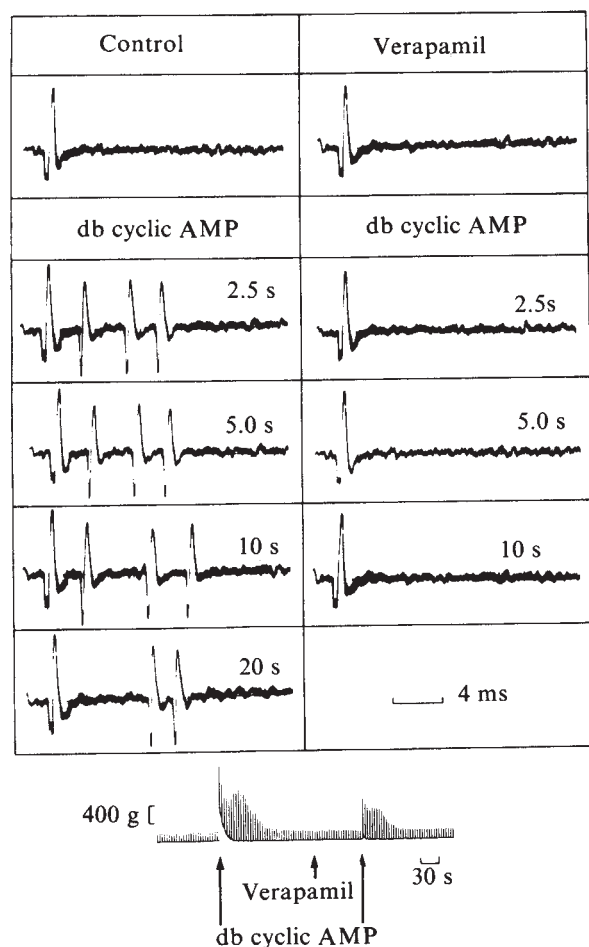


Fig. 1 Top, effect of verapamil on SBR produced in a soleus motor axon by db cyclic AMP. On the left, the first trace shows the response to a single stimulus applied before either drug; subsequent traces show the responses to similar stimuli applied once every 2.5 s after the intra-arterial administration of db cyclic AMP ($200\text{ }\mu\text{g kg}^{-1}$). On the right, the first trace shows the response of a single stimulus applied after the intra-arterial administration of verapamil ($0.1\text{ }\mu\text{g kg}^{-1}$) and subsequent traces show the responses of similar stimuli to db cyclic AMP following verapamil administration. Bottom, effects of verapamil on the potentiation of soleus muscle force produced by db cyclic AMP. Left, response produced by db cyclic AMP ($200\text{ }\mu\text{g kg}^{-1}$) on a neurally-stimulated muscle. Right, response of the same preparation to db cyclic AMP after administration of verapamil ($0.1\text{ }\mu\text{g kg}^{-1}$).

the SBR produced by effects of db cyclic AMP or NaF suggests that sodium flux is not responsible for this latter phenomenon. Its sensitivity to the calcium antagonists lanthanum and verapamil, however, suggests that the SBR which accompanies the administration of db cyclic AMP or NaF is mediated by a calcium current.

Since a prolongation of the negative after-potential of the nerve terminal is implicit in this explanation for the generation of repetitive activity by db cyclic AMP and NaF, there are several mechanisms by which calcium influx would serve this end. With regard to a direct mechanism, the influx of calcium is known to carry a late depolarising current in nerves¹⁵ and prolongation of this current by cyclic AMP could directly affect the afternegativity of the nerve terminal. An indirect mechanism is also possible since it has been shown that inward calcium currents activate late potassium conductance in neurones and that this calcium-dependent potassium conductance produces an after-hyperpolarisation¹⁶⁻¹⁸. Hyperpolarisation of the nerve terminal can set up conditions wherein the negative after-potential of the invading action potential is prolonged enough to generate SBR¹⁹. This possibility is given further support by reports that theophylline potentiates the after-hyperpolarisation in bullfrog sympathetic ganglia and that

Fig. 2 Top, effect of lanthanum chloride on SBR produced in a soleus motor axon by NaF. Left, first trace is the response to a single stimulus applied before either drug; subsequent traces show the responses to similar stimuli applied once every 2.5 s after the intra-arterial administration of NaF ($100 \mu\text{g kg}^{-1}$). Right, first trace is the response to a single stimulus applied after lanthanum chloride administration ($0.01 \mu\text{g kg}^{-1}$); subsequent traces show the responses to NaF following the administration. Bottom, effects of lanthanum chloride on the potentiation soleus muscle force produced by NaF. Left, response produced by the administration of NaF ($100 \mu\text{g kg}^{-1}$) to neurally-stimulated muscle. Right, response to NaF after the administration of lanthanum ($0.01 \mu\text{g kg}^{-1}$).

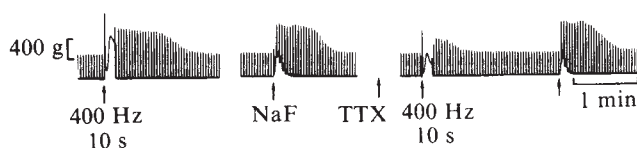
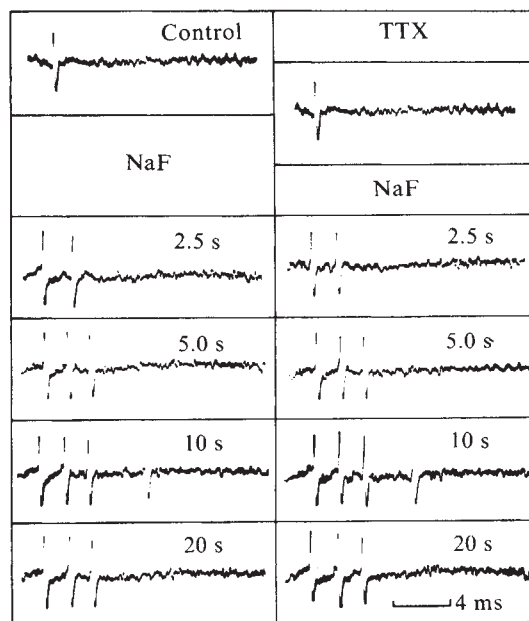
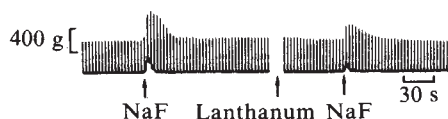
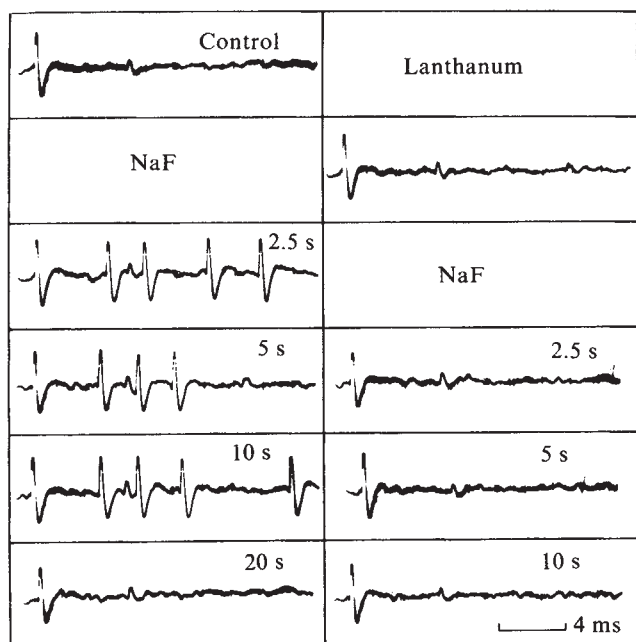


Fig. 3 Top, Effect of TTX on the SBR produced in a soleus motor axon by the administration of db cyclic AMP. On the left, the first trace shows the response to a single stimulus applied before either drug. Subsequent traces show the responses to similar stimuli applied at the noted intervals after the administration of db cyclic AMP ($200 \mu\text{g kg}^{-1}$). On the right, the first trace shows the response to a single stimulus applied after the intra-arterial administration of TTX; subsequent traces show the responses to db cyclic AMP following the administration of TTX. Bottom, Effects of TTX on PTR and the potentiation of soleus muscle force produced by db cyclic AMP. Left, Responses produced by high frequency stimulation (400 Hz for 10 s) and by administration of db cyclic AMP ($200 \mu\text{g kg}^{-1}$ intra-arterially). Right, Response of the same preparation to high frequency stimulation and db cyclic AMP after administration of TTX ($0.01 \mu\text{g kg}^{-1}$).

this effect is abolished by the removal of extracellular calcium²⁰.

The conclusion that intraneuronal cyclic AMP induces an influx of calcium is also of interest because an influx of calcium into nerve endings is known not only to carry a depolarising current and increase potassium conductance, but also to trigger the release of transmitter²¹. This suggests that cyclic nucleotides might be important in transmitter release.

In summary, our observations indicate that db cyclic AMP and related reagents produce repetitive activity in motor axons by promoting the influx of calcium into the motor nerve terminal and suggest that a cyclic nucleotide system may be part of the mechanism that regulates calcium flux into motor nerve terminals.

This work was supported by a grant from the US Public Health Service.

L. R. SKIRBOLL*
L. BAIZER
K. L. DRETCHEN

Department of Pharmacology,
Georgetown University Schools of
Medicine and Dentistry,
3900 Reservoir Road,
Washington, DC 20007

Received 3 January; accepted 23 May 1977.

*Present address: Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510.

- ¹ Dretchen, K. L., Standaert, F. G., Skirboll, L. R. & Morgenroth, V. H., III *Nature* **264**, 79–81 (1976).
- ² Standaert, E. G., Dretchen, K. L., Skirboll, L. R. & Morgenroth, V. H., III *J. Pharmac. exp. Ther.* **199**, 544–552 (1976).
- ³ Standaert, E. G., Dretchen, K. L., Skirboll, L. R. & Morgenroth, V. H., III *J. Pharmac. exp. Ther.* **199**, 553–564 (1976).
- ⁴ Fleckenstein, A. & Grun, G. *Pflügers Arch. Ges. Physiol.* **307**, R26 (1969).
- ⁵ Baker, P. F., Meves, H. & Ridgway, E. B., *J. Physiol., Lond.* **231**, 527–548 (1973).
- ⁶ Takata, M., Pickard, W. F., Lettvin, J. Y. & Moore, J. W. *J. Physiol., Lond.* **50**, 461–471 (1966).
- ⁷ Riker, W. F., Roberts, J., Standaert, F. G. & Fugimori, H. *J. Pharmac.* **211**, 286–312 (1957).
- ⁸ Ritchie, J. M. & Straub, R. W. *J. Physiol., Lond.* **136**, 80–97 (1975).
- ⁹ Standaert, F. G. *J. gen. Physiol.* **47**, 53–70 (1963).
- ¹⁰ Standaert, F. G. & Riker, W. F. *Ann. N.Y. Acad. Sci.* **144**, 517–533 (1967).
- ¹¹ Berridge, M. J. in *Advances in Cyclic Nucleotide Research* 6 (eds Greengard, P. & Robison, G. A.) 1–98 (Raven, New York 1975).
- ¹² Rudolph, S. A. & Greengard, P. *J. biol. Chem.* **249**, 5684–5687 (1974).
- ¹³ Greengard, P. & Kebedjian, J. W. *Fedn Proc.* **33**, 1059–1066 (1974).
- ¹⁴ Rasmussen, H., Jensen, P., Lake, W., Friedman, N. & Goodman D. P. B. in *Advances in Cyclic Nucleotide Research* 5 (eds Greengard, P. & Robison, G. A.) 375–394 (Raven, New York, 1975).
- ¹⁵ Baker, P. F., Hodgkin, A. L. & Ridgway, E. B. *J. Physiol., Lond.* **218**, 709–755 (1971).
- ¹⁶ Meech, R. W. *Comp. Biochem. Physiol.* **42A**, 493–499 (1972).
- ¹⁷ Meech, R. W. & Standen, N. B. *J. Physiol., Lond.* **249**, 211–239 (1975).
- ¹⁸ Barrett, E. F. & Barrett, J. N. *J. Physiol., Lond.* **255**, 737–774 (1976).
- ¹⁹ Ritchie, J. M. & Straub, R. W. *J. Physiol., Lond.* **134**, 698–711 (1956).
- ²⁰ Busis, N. A. & Weight, F. F. *Nature* **263**, 436–437 (1976).
- ²¹ Hubbard, J. I. *Physiol. Rev.* **674**–723 (1973).

A modifier of (Na⁺ + K⁺) ATPase in commercial ATP

WHEN most (Na⁺ + K⁺)ATPase preparations are allowed to hydrolyse ATP in the presence of different concentrations of Na⁺ and K⁺ ions, but with the sum [Na⁺] plus [K⁺] held constant, the behaviour observed is similar to that shown in the dotted curve of Fig. 1. K⁺ ions activate at low concentrations, and inhibit (by competing with Na⁺ ions at intracellular sites) at high concentrations. There is a broad plateau in the middle of the curve. We reported¹ recently that (Na⁺ + K⁺)ATPase from the outer medulla of pig kidney showed behaviour of a different kind, like that shown in the solid curve of Fig. 1. Here, K⁺ ions apparently inhibit even at relatively low concentrations, so that, as the activating effect of K⁺ ions approaches saturation, the inhibitory effect becomes predominant and causes a sharp fall to an intermediate level of activity. This effect was more pronounced at 20 or 30 °C than at 37 °C, and at pH 7.5 than at pH 6.5. We have now discovered that this peculiar behaviour depends on the use of Sigma 'Sigma grade' ATP, and is not seen with Boehringer ATP (see Fig. 1). We report here that many samples of 'Sigma grade' ATP contain something which reacts with (Na⁺ + K⁺)ATPase reversibly and with a high affinity, making the enzyme much more sensitive to inhibition by K⁺ ions. The changed response to K⁺ ions, and the sensitivity of this response to pH can account for the inhibition of kidney (Na⁺ + K⁺)ATPase by 'Sigma grade' ATP reported by Charney, Silva and Epstein², and the altered pH optimum reported by Katz and Michell³.

Evidence that it is the Sigma 'Sigma grade' ATP that carries the modifying agent comes from experiments of two kinds. First an experiment like that of Fig. 1 but using an equimolar mixture of Sigma 'Sigma grade' and Boehringer ATP gave the Sigma pattern. Since the modifier must have been present in the mixture, it is evidently the Sigma pattern that represents the behaviour of the modified enzyme. An alternative approach, using mixtures of Sigma and Boehringer ATP in different proportions, is illustrated in Fig. 2. Here the total ATP concentration was held constant at 3 mM or 6 mM, [Na⁺] and [K⁺] were fixed at 130 mM and 20 mM respectively, and [Mg²⁺] was equal to the total ATP concentration. It is important to note that 3 mM ATP was sufficient to saturate the enzyme. The results show that, in these conditions, the activity was highly sensitive to the level of Sigma ATP but was scarcely affected by the level of Boehringer ATP. This implies (1) that it is the Sigma 'Sigma grade' ATP that carries the modifier, and (2) that the modifier is not competitive with ATP. Separate

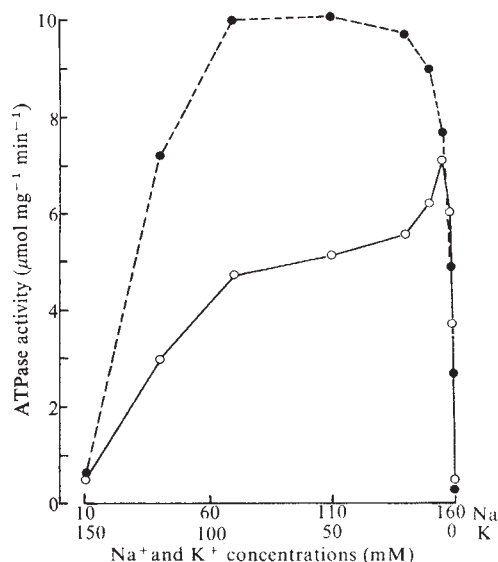


Fig. 1 Rate of ATP hydrolysis as a function of Na⁺ and K⁺ concentrations. (Na⁺ + K⁺)ATPase prepared from the outer medulla of pig kidney by the simpler method of Jørgensen⁶ was incubated at 37 °C for 20 min in a medium containing 2.5 mM ATP, 2.5 mM MgCl₂, 30 mM histidine (pH 7.5), 0.1 mM EGTA, and NaCl and KCl to give the concentrations shown on the abscissa. The amount of ATP hydrolysed by the end of the incubation did not exceed 10% of the total. The rate of hydrolysis for each set of conditions was estimated from the orthophosphate released after 20 min, as preliminary experiments showed that deviations from linearity during this period could be neglected. ●, Boehringer ATP; ○, 'Sigma grade' ATP.

experiments (not shown) indicate that the inhibition by Sigma 'Sigma grade' ATP is reversible and is not affected by changes in the enzyme concentration. The results in Fig. 2 may therefore be used to calculate a 'K_i' for Sigma 'Sigma grade' ATP, which works out as 2.18 mM from the data at 3 mM total ATP and 2.39 mM from the data at 6 mM total ATP. If the modifier is present at a molar concentration of, say, 1% of the Sigma ATP, its K_i must be about 23 μM. The figure of 1% is, of course, entirely arbitrary, but it is clear that, on any likely assumptions, the affinity of the modifier for the enzyme must be high.

The most obvious questions raised by these findings are: what is the modifier, and, since Sigma 'Sigma grade' ATP is extracted from horse muscle, does it have a physiological role? The modifier has been present in most, but not all, samples of Sigma 'Sigma grade' ATP that we have used over the past year, and is not restricted to a single lot number. So far we have not succeeded in separating the modifier from the ATP. Passing Sigma 'Sigma grade' ATP down a cation exchange column in the H⁺ form did not alter its subsequent behaviour, so that if the modifier is a cation it must be very tightly bound to the ATP. When the ATP was adsorbed on to a column of Dowex 1 resin in the Cl⁻ form, the modifier was not eluted by a volume of 20 mM NH₄Cl–20 mM HCl sufficient to elute most of the contaminating ADP, but was eluted along with ATP by 250 mM HCl. Clearly, more sophisticated chromatography is necessary. An alternative approach to identifying the modifier is to think of possible candidates and add them individually to 3 mM Boehringer ATP. GTP, CTP, UTP, deoxyATP, adenosine tetraphosphate, 2,3-diphosphoglycerate, fructose 1,6-diphosphate and Ca²⁺ ions have been tested in this way, but none gave the Sigma pattern.

Another interesting question is: what does the modifier do to the enzyme that changes its response to K⁺ ions so dramatically? Inhibition by K⁺ ions at low concentrations is a feature of the peculiar (Na⁺ + K⁺)ATPase found in low-potassium ungulate red cells, and in these cells the K⁺ ions

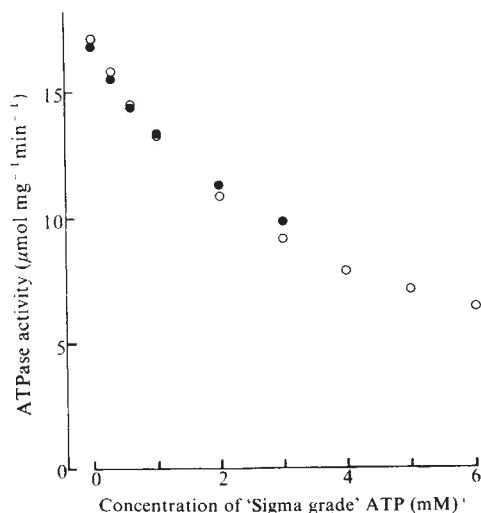


Fig. 2 The effect of substituting Sigma 'Sigma grade' ATP for Boehringer ATP on the rate of ATP hydrolysis by $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ from pig kidney outer medulla. The enzyme was incubated for 20 min at 37°C in a medium containing 3mM (●) or 6 mM ATP (○), MgCl_2 equimolar with the ATP, 20mM KCl, 130 mM NaCl, 30 mM histidine (pH 7.5) and 0.1 mM EGTA.

act intracellularly⁴. With kidney $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ it is not possible to tell at which surface of the membrane the K^+ ions act. We hope to resolve this problem by using resealed human red cell ghosts, for we have found that the human red cell $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ behaves like the solid curve of Fig. 1 when hydrolysis of Sigma 'Sigma grade' ATP containing the modifier is allowed to occur at subnormal temperatures. It is also intriguing that the effect of the modifier on the kidney $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ is entirely prevented if the enzyme is first incubated with trypsin in the absence of K^+ ions, until the rapid phase of inactivation (see ref. 5) is complete. The residual enzymatic activity behaves like the dotted curve in Fig. 1 whether Sigma 'Sigma grade' ATP or Boehringer ATP is used (ref. 1 and unpublished).

We thank Drs P. L. Jørgensen, P. A. Merton, and J. D. Cavieser for helpful advice. This work was supported by the MRC.

L. A. BEAUGÉ*
I. M. GLYNN

Physiological Laboratory,
University of Cambridge,
Cambridge, UK

Received 25 April; accepted 9 June 1977.

*Present address: Department of Biophysics, University of Maryland School of Medicine, Baltimore, Maryland 21201.

¹ Beaugé, L. A. & Glynn, I. M. *J. Physiol., Lond.* **265**, 41P–42P (1977).

² Charney, A. N., Silva, P. & Epstein, F. H. *J. appl. Physiol.* **39**, 156–158 (1975).

³ Katz, A. I. & Michell, A. R. *J. Physiol., Lond.* **263**, 210P–211P (1976).

⁴ Glynn, I. M. & Ellory, J. C. in *Role of Membranes in Secretory Processes* (eds Bolis, L., Keynes, R. D. & Wilbrandt, W.) 224–237 (North-Holland, Amsterdam, 1972).

⁵ Jørgensen, P. L. *Biochim. biophys. Acta* **401**, 399–415 (1975).

⁶ Jørgensen, P. L. *Biochim. biophys. Acta* **356**, 36–52 (1974).

The molecular mechanisms of anaesthesia

It is generally accepted that neutral anaesthetics, such as the *n*-alkanols and *n*-alkanes, act from a hydrophobic site¹. Discussion has tended to focus on the nature of this site and, particularly, whether it is located in a membrane protein or in the interior of a bilayer^{2,3}. If the site is in a bilayer then the question arises as to how the proteins involved in nervous impulse propagation are affected. We present here new evidence concerning the anaesthetic properties of the

n-alkanes which points to the bilayer as the site of action and also suggests a mechanism for the inhibition of impulse propagation. Briefly, the cut-off in anaesthetic potency on ascending the homologous series is found to be closely related to the decrease in adsorption of the alkane into a cholesterol-containing bilayer. In addition, the anaesthetic hydrocarbons produce a concentration-dependent increase in bilayer thickness. On the basis of these two observations, and from our knowledge of the properties of the ion channel-forming polypeptide gramicidin A (ref. 4) we propose that a thickening of the bilayer regions of the nerve membrane by the alkanes destabilises the open ionic channels formed during electrical excitation.

Several factors make the *n*-alkanes of particular interest in the study of anaesthesia. First, they do not have multiple functional groups which would complicate analyses of their interactions. Second, their interactions with bilayers are relatively well understood from their use as solvents in the formation of black lipid films⁵. Finally, they constitute a homologous series in which there is a hitherto unexplained loss in anaesthetic potency of the higher members^{1,6}. This cut-off effect was first revealed by the experiments of Fühner^{7,8} on general anaesthesia in mice and has been confirmed by Mullins, who showed that mice are unaffected by *n*-decane even after prolonged exposure⁶. We have investigated the ability of *n*-alkanes to block the propagation of the nervous impulse in isolated nerve preparations. Figure 1 shows that *n*-pentane and *n*-hexane cause rapid blockage of the action potential in both squid giant axons and in frog sciatic nerve. Although the information is not strictly required for present purposes, voltage-clamp experiments (to be described elsewhere) show that both the sodium and, to a lesser extent, the potassium currents are reduced by the alkane. For short exposure times and incomplete suppression of the action potential, the blockages were normally reversible. *n*-Nonane and *n*-decane have no significant effect in either preparation. These results are not accounted for by low solubilities and long diffusion times. The new data show, therefore, that as in whole animals, there is a marked decline in anaesthetic potency with increasing chain length in the *n*-alkanes between pentane and nonane.

The adsorption of *n*-alkanes into a lipid bilayer can be measured using black film techniques^{5,15}. The bilayers are formed so as to be in equilibrium with solutions of the alkane under examination. Measurements of the electrical capacity per unit area yield the thickness of the hydrocarbon region of the bilayer and, since the areas per molecule of the lipids (for example, phospholipid and cholesterol) present are, at most, weakly dependent on the alkanes used, changes in capacity reflect changes in alkane adsorption. Previous studies have demonstrated an effect of chain length on adsorption, but only for molecules larger than *n*-decane⁹. But, these results were obtained for bilayers which did not contain cholesterol. Analyses of nerve membranes indicate that their cholesterol-phospholipid ratio is approximately 1:3 (ref. 10) and consequently the alkane adsorption experiments were repeated with bilayers of composition comparable with this. Figure 2 shows that the chain length dependence of adsorption is now between *n*-pentane and *n*-decane and, from the thicknesses, it can be concluded that for homologues larger than decane there is very little alkane in the bilayer⁹.

The close correlation between the decline in impulse blocking potency and the decrease in bilayer adsorption for the *n*-alkanes, revealed by Figs 1 and 2, would not have been predicted from the bulk phase partition coefficients as in the Meyer–Overton hypothesis¹, and constitutes a simple, if superficial, explanation for the chain length cut-off effect. Moreover, since it is known that the *n*-alkanes accumulate primarily in the centre of a bilayer¹¹, the correlation reinforces the idea that it is from this position that they exert their anaesthetic action. It is, of course, quite possible that hydrophobic binding sites in membrane proteins may exhibit a size discrimination similar to that of the lecithin–cholesterol bilayer but, until this is demon-

strated, the protein site hypothesis is not the simplest available. In a non-equilibrium system it could be argued that even if the protein itself were the site of action and showed no chain length discrimination, a cut-off effect might still be seen if access to the protein were through the lipid bilayer. But, the experimental evidence outlined earlier does not support the notion that the chain length cut-off is a non-equilibrium effect. Hence the route which the anaesthetic might take to a target does not have to be considered.

It may be significant that a similar chain length dependence exists for both impulse blockage within an axon and for general anaesthesia by the alkanes. Thus, even though it seems likely that different proteins are involved in these two processes, they

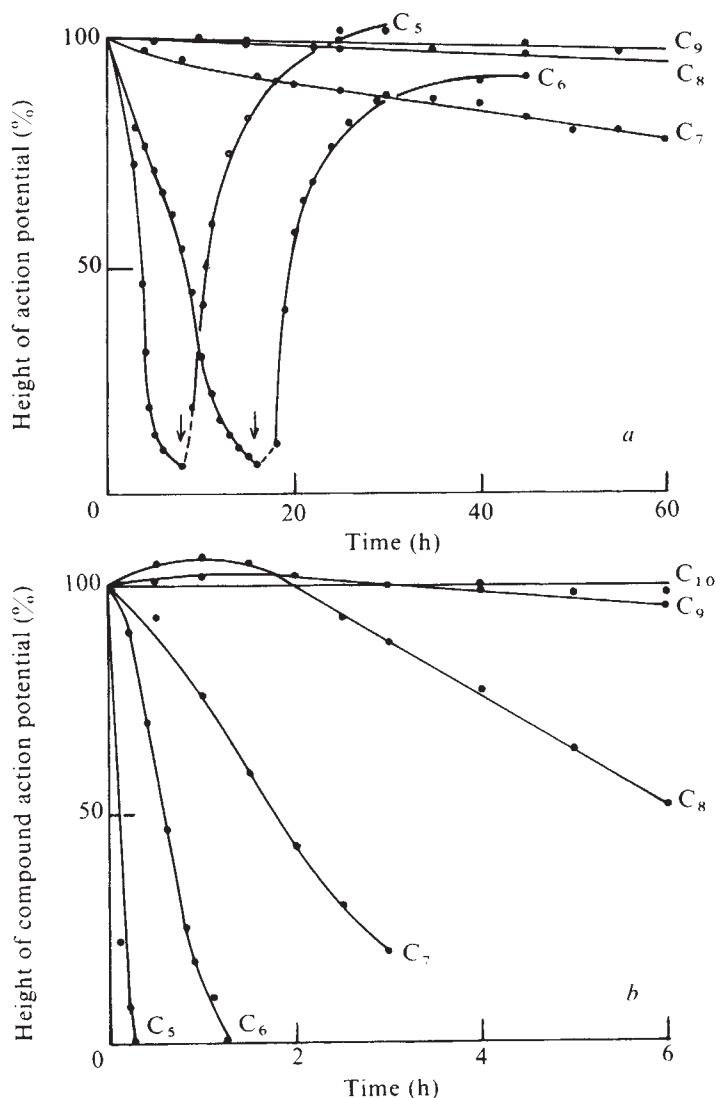


Fig. 1 Nerve impulse blockage by the *n*-alkanes from *n*-pentane (C₅) to *n*-decane (C₁₀). *a*, The propagated action potential of the squid giant axon (from *Dorytheutis plei*) monitored as the chamber was perfused with saturated solutions of the alkanes in artificial seawater. Vertical arrows indicate a switch to alkane-free seawater. Flow rates were approximately 10 ml min⁻¹; internal recording was with a 0.5 M KCl-filled glass capillary; the temperature was 18 °C. The effect of the alkanes on the resting potential of the axon was small and variable. *b*, The compound action potential of the desheathed frog sciatic nerve from *R. temporaria*. Dilute emulsions of alkanes in frog Ringer were used to ensure an adequate supply of material; saturated solutions became depleted by a large uptake into the nerve bundle. Perfusion rates were about 1.5 ml min⁻¹. The nerve was stimulated at 6 Hz and monophasic compound action potentials were recorded from a platinum electrode situated at the end of the nerve. The temperature was 18–21 °C.

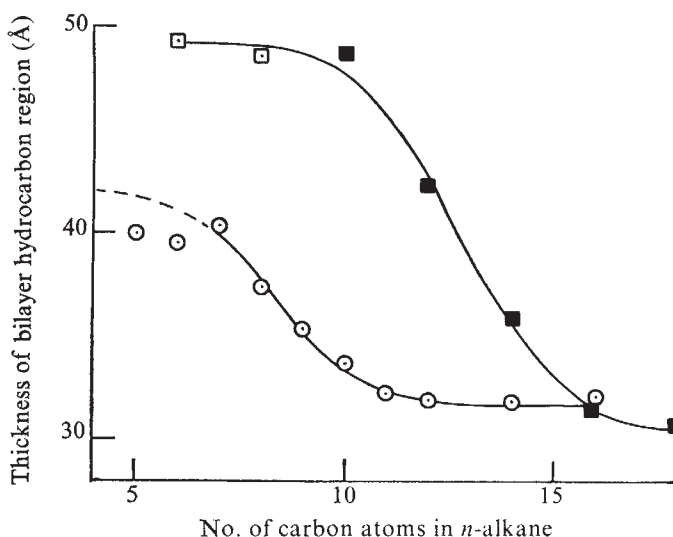


Fig. 2 The variation in hydrocarbon layer thickness of black lipid films formed in equilibrium with different *n*-alkanes. ○, Egg phosphatidylcholine-cholesterol (~2.5:1) films; the bulk lipid solution was 7 mM phospholipid and 21 mM cholesterol. The pentane and hexane results are possibly low owing to the difficulty of preventing evaporation of these volatile solvents. ■, Cholesterol-free egg phosphatidylcholine films. (■, data of ref. 9). The aqueous phase was 0.1 M NaCl and the temperature was 20 °C. In each experiment the lipids were dissolved in the appropriate alkane and the aqueous solution was equilibrated with the lipid solution before the start of the experiment.

would need to have comparable alkane binding properties. While this is quite conceivable, it is simpler for the moment to invoke the ubiquity of the lipid bilayer.

The supposition that nerve impulse blockage originates in a lipid bilayer raises the question of exactly how the mechanisms of ion movement involved in electrical excitability might be affected. Recent evidence concerning the unit conductances of ion channels in excitable tissues^{12–14} supports the notion that these channels consist of pore-like structures. In view of this, it is of interest to consider how the functioning of a relatively well-characterised polypeptide pore, such as gramicidin A, is affected by modification of its bilayer environment. It is known that the conductance of a black lipid film containing gramicidin decreases rapidly as the thickness and hydrocarbon content of the film increases^{4,15}. This arises from a lowering of the stability of the open channels. Since bilayer thickness is increased by the *n*-alkanes (Fig. 2) these observations may have some relevance to anaesthesia. An empirical relationship for the constant *K* of equilibrium between open and closed gramicidin channels in a black lipid film can be written in the form

$$K \approx A \exp(-h/B)$$

where *h* is the thickness of the membrane and is considered to take values only larger than or equal to the length of the gramicidin channel. *A* is a constant, and *B*, for the particular system and range of *h* concerned, is 2.2 Å (ref. 15). This equation is consistent with the proposal that a dimpling of the lipid is required for the functioning of a pore in a membrane of thickness greater than the pore length (Fig. 3). The dimpling involves an increase in the surface area of the bilayer and hence requires an investment of energy proportional to the surface tension. On the basis of this crude model, therefore, the free energy of the open pore should be roughly proportional to the bilayer thickness, so giving the observed exponential form of the above equation. In addition, the value of the bilayer tension which corresponds to *B* = 2.2 Å is approximately 3 mN m⁻¹, in good agreement with experiment^{9,16}.

Provided that the *n*-alkanes increase the thickness of bilayer

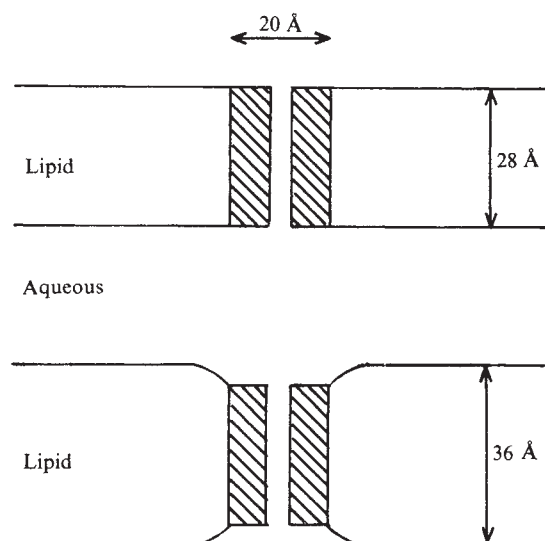


Fig. 3 The thickness-tension model. The shaded region represents a gramicidin channel situated in a lipid bilayer. In the top diagram the bilayer is of thickness equal to the channel length. In the lower diagram the bilayer thickness has been chosen to correspond with the concentration of pentane (about 90% saturated) sufficient to suppress completely the action potential in the nerve axon. The deformation or dimpling of the bilayer in the lower diagram involves a surface free energy change which tends to reduce the stability of the open channel.

regions surrounding the ion channels in excitable membranes, then the type of mechanism proposed for gramicidin could obviously apply also to impulse blockage. Whether or not the channels in an axon are of dimensions comparable with those shown in Fig. 3 is of no great importance for the general argument. Thus, if a channel is normally in equilibrium with the surrounding membrane, a thickness change in the lipid is almost certain to stress the channel and hence destabilise it. While there is no conclusive evidence that the *n*-alkanes do thicken biological membranes, there are strong indications that this is so. First, measurements of the adsorption of *n*-alkanes into erythrocyte membranes have given results which are very similar in both their chain length dependence and in their absolute values to the data of Fig. 2 for cholesterol-containing bilayers (F. Dagger and D.A.H., in preparation). Second, preliminary experiments on the squid giant axon at its normal resting potential, suggest that its capacity per unit area, measured at 1 kHz and with 1 mV applied, decreases by about 15% in pentane-saturated artificial seawater (D.A.H., J. Kimura and B. Urban, unpublished). Circumstantial evidence therefore supports the proposed thickness-tension link between the perturbation of bilayer structure by the alkanes and their anaesthetic potency.

There are no compelling reasons for believing that all anaesthetics share a common mechanism, but it seems possible that the bilayer thickness-tension model outlined above may apply to some more polar anaesthetics. For example, a cut-off in potency with increasing molecular size occurs for the *n*-alkanols¹⁷ and for the barbiturates¹⁸, implying that they also may act through thickening of a bilayer. Furthermore, ethanol and diethyl ether at anaesthetic concentrations both lower the capacity per unit area of black films and therefore must increase the film thickness. Finally, the reversal of anaesthesia by the application of pressure, which has been reported for a range of systems, is thought to take place through the restoration of a hydrophobic region, expanded by the anaesthetic, to its original dimensions¹⁹. If this is so, it is consistent with the idea of membrane thickness changes and with the existence of a common mechanism for a variety of molecules.

After submitting this paper we learnt of a paper by R. G.

Ashcroft, H. G. L. Coster and J. R. Smith (*Nature*, in the press) in which it was reported that benzyl alcohol caused a thickening of lipid bilayers, and in which it was suggested that this effect might be instrumental in inhibiting sodium channel conductance.

D.A.H. acknowledges support from the SRC.

D. A. HAYDON
B. M. HENDRY
S. R. LEVINSON*

*Physiological Laboratory,
University of Cambridge,
Downing Street, Cambridge, UK*

J. REQUENA

*Centro de Biofisica,
Instituto Venezolano de Investigaciones Cientificas,
Apartado 1827, Caracas 101, Venezuela*

Received 10 February; accepted 2 June 1977.

*Present address: 164-30, Division of Chemistry, California Institute of Technology, Pasadena, California.

- 1 Seeman, P. *Pharmac. Rev.* **24**, 583-655 (1972).
- 2 Boggs, J. M., Yoong, T. & Hsia, J. C. *Molec. Pharmac.* **12**, 127-135 (1976).
- 3 Lee, A. G. *Nature* **262**, 545-548 (1976).
- 4 Haydon, D. A. & Hladky, S. B. *Q. Rev. Biophys.* **5**, 187-282 (1972).
- 5 Fettiplace, R. et al. in *Meth. membr. Biol.* **4** (ed. Korn, E. D.) 1-75 (Plenum, New York, 1975).
- 6 Mullins, L. J. in *Handbook of Neurochemistry* **6** (ed. Lajtha, A.) 395-421 (Plenum, New York, 1971).
- 7 Fühner, H. *Biochem. Z.* **115**, 235-261 (1921).
- 8 Ferguson, J. *Proc. R. Soc. B127*, 387-404 (1939).
- 9 Fettiplace, R., Andrews, D. M. & Haydon, D. A. *J. membr. Biol.* **5**, 277-296 (1971).
- 10 Chacko, G. K., Villegas, G. M., Barnola, F. V., Villegas, R. & Goldman, D. E. *Biochim. biophys. Acta* **443**, 19-32 (1976).
- 11 Andrews, D. M., Manev, E. D. & Haydon, D. A. *Spec. Disc. Faraday Soc.* **1**, 46-56 (1970).
- 12 Rang, H. P. *Q. Rev. Biophys.* **7**, 283-399 (1975).
- 13 Almers, W. & Levinson, S. R. *J. Physiol., Lond.* **247**, 483-509 (1975).
- 14 Conti, F., DeFelice, L. J. & Wanke, E. *J. Physiol., Lond.* **248**, 45-82 (1975).
- 15 Haydon, D. A. *Ann. N.Y. Acad. Sci.* **268**, 2-16 (1975).
- 16 Requena, J. & Haydon, D. A. *Proc. R. Soc. A347*, 161-177 (1975).
- 17 Brink, F. & Posternak, J. M. *J. cell. comp. Physiol.* **32**, 211-233 (1948).
- 18 Mautner, H. G. & Clemson, H. C. in *Medicinal Chemistry*, 3rd edn (ed. Burger, A.) 1365-1383 (Wiley-Interscience, New York, 1970).
- 19 Miller, K. W., Paton, W. D. M., Smith, R. A. & Smith, E. B. *Molec. Pharmacol.* **9**, 131-143 (1973).

Membrane asymmetry and blood coagulation

ALTHOUGH there is compelling evidence that membrane components are non-randomly distributed between the two surfaces, the physiological significance of membrane asymmetry has so far remained obscure. Since the exterior surface of blood cell membranes is presumably devoid of negatively charged phospholipids which have a regulatory role in blood clotting, a possible function of lipid asymmetry in the coagulation process can be anticipated. We describe here experiments devised to show that the asymmetric distribution of membrane phospholipids in blood cells may serve a biological purpose, by contributing to maintain the delicate balance between regulating haemostasis and avoiding thrombosis.

The main function of phospholipids in blood coagulation is to provide a catalytic surface on which various coagulation factors interact, thus increasing their local concentrations. For example, the final lipid-activated step in the coagulation process is the conversion of prothrombin into thrombin by the prothrombinase complex, which requires at least four components in addition to prothrombin, the proteins factor X_a , and factor V_a , Ca^{2+} and phospholipids¹. Of these components, only factor X_a is able to convert prothrombin into thrombin by limited proteolysis¹⁻⁵, but its individual action is considerably slower than with the complete prothrombinase complex⁶.

In situ, the platelet plasma membrane presumably provides the phospholipid-water interface (platelet factor 3), which is required to accelerate the coagulation process⁷. It has been shown⁸⁻¹⁰ that the clot-promoting activity of phospholipids *in vitro* is not attributable to a certain phospholipid class, but to a specific negative charge of the phospholipid surface. In particular, negatively charged phospholipids such as phosphatidylserine and phosphatidylglycerol (when properly diluted

with neutral phospholipids like lecithin or phosphatidyl ethanolamine) exhibit maximal activation of the coagulation-process^{11,12}.

It is generally accepted that membranes are highly asymmetric structures both with respect to their proteins and their lipids (see refs 13–16 for recent reviews). We have shown that the non-random distribution of phospholipids between the interior and the exterior half of the platelet plasma membrane is similar to that of the erythrocyte membrane¹⁷. In both membranes, phosphatidylserine is apparently nearly exclusively located on the cytoplasmic surface, whereas the outer monolayer of the membrane consists of neutral phospholipids, particularly sphingomyelin. The other two major phospholipid classes, lecithin and phosphatidylethanolamine, are present on both membrane sides though not to the same extent. We report here on the possible implications of blood cell membrane asymmetry for the coagulation process. Since methods are readily available to prepare different types of sealed and non-sealed erythrocyte ghosts, this type of membrane has been used in the present study rather than the platelet surface membrane which is more difficult to manipulate.

Blood from healthy volunteers was collected in 0.13 M sodium citrate (9 ml blood + 1 ml citrate). Red cells, non-sealed ghosts and resealed right-side out ghosts were prepared as described previously¹⁸. Inside-out sealed ghost vesicles were prepared according to Steck¹⁹. The coagulant activity of these preparations was determined by its ability to shorten the clotting time on recalcification of 'normal' human platelet-poor plasma in the presence of Russell's viper venom (legend to Fig. 1). For the presentation of the coagulant activity of membranes and lipids we report directly the clotting times rather than attempt to convert these to some arbitrary units of activity. It is realised

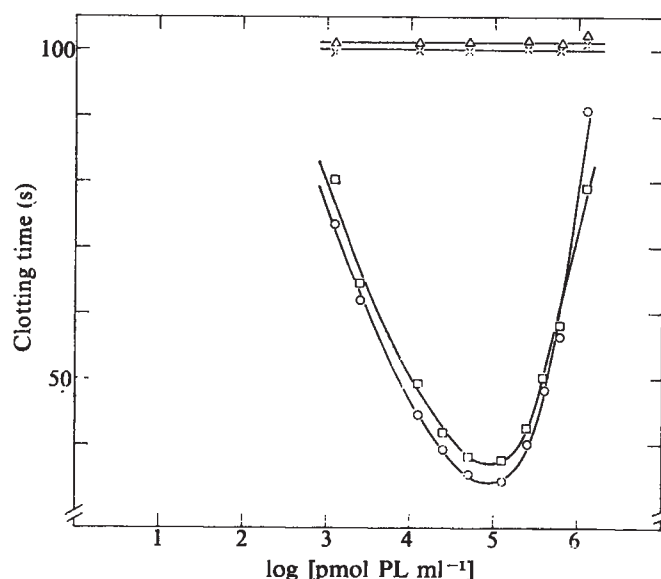


Fig. 2 Effect of liposomes on clotting time of plasma. Liposomes simulate inner and outer half of red cell and platelet plasma membranes. $\times$, Platelet surface membrane outer phospholipids; $\square$, platelet surface membrane inner phospholipids; Δ , red cell membrane outer phospholipids; $\circ$, red cell membrane inner phospholipids. For phospholipid compositions see Table 1. Assay conditions as for Fig. 1, phospholipid added as liposomes.

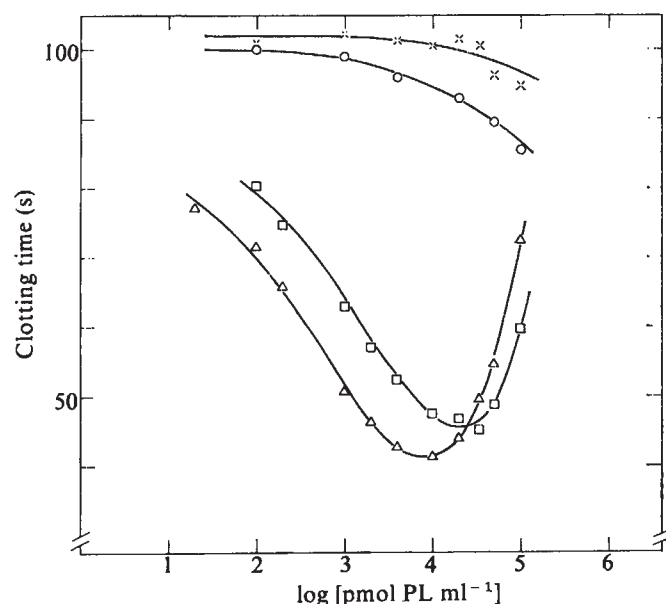


Fig. 1 Effect of in- and outside of erythrocyte membranes on clotting time of plasma. Assay: 0.1 ml of citrated plasma (centrifuged 10 min, 5000g) was incubated at 37 °C for 30 s with 12 ng of Russell's viper venom (which gives a blank coagulation time of 100 s on recalcification), followed by addition of 0.1 ml of various dilutions of red cells or ghosts and 0.1 ml 25 mM CaCl_2 . A stopwatch was started simultaneously with the addition of CaCl_2 . The time required for the formation of a firm fibrin clot was determined by the arrest of a rotating metal wire (2 mm length, cut from a regular paper clip), driven by a magnetic stirrer. This method gives the same clotting times as observed by using a Kollie hook, but the standard deviations are smaller and usually below 1%. PL, phospholipid added as red cells or ghosts. $\times$, Erythrocytes; $\circ$, resealed ghosts; Δ , inside-out vesicles; $\square$ non-sealed ghosts.

that such clotting times are a complex function of lipid activity, but no real advantage is gained by using conventional log-log calibration curves obtained by serial dilution of plasma. In view of the wide range of the final membrane phospholipid concentration, the values on the abscissa are plotted logarithmically.

When only the outside of the erythrocyte membrane is exposed (either intact cells or resealed ghosts) no significant reduction of the blank clotting time is observed over a wide range of concentration (Fig. 1). On the other hand, when either the membrane interior (inside-out vesicles) or both membrane sides (non-sealed ghosts) are exposed, a two- to threefold reduction of the coagulation time is observed at optimal concentrations. At higher ghost concentrations, clotting times increase; this is consistent with the view that excess of lipid will result in a dilution of coagulation factors at the lipid surface²⁰. The results suggest that only the phospholipids at the membrane interior have procoagulant activity, also since inside-out ghost vesicles are slightly more active than non-sealed ghosts.

This hypothesis was further investigated by testing liposomes with the same phospholipid composition as present in the outer half and inner half of human red cell membranes and pig platelet plasma membranes (Table 1). Handshaken liposomes (4 μmol phospholipid per ml of 0.9% (w/v) NaCl) were used

Table 1 Mole ratios of phospholipid in liposomes simulating outer and inner half of red cell and platelet plasma membranes

	Red cell membrane		Platelet plasma membrane	
	Outer half	Inner half	Outer half	Inner half
PC	44	15	26	34
Sph	44	10	52	4
PE	12	47	20	28
PS	0	28	2	34

Data calculated from refs 17 and 21. PC, phosphatidylcholine prepared from egg yolk²²; Sph, sphingomyelin prepared from bovine brain²³; PE, phosphatidylethanolamine prepared from egg yolk²²; PS, phosphatidylserine prepared from pig brain²⁴.

rather than sonicated vesicles, to avoid artificially-induced phospholipid asymmetry which is known to occur in mixed vesicles as a result of charge differences and packing properties²⁵. It should be noted, however, that with multilamellar liposomes only 10–15% of the lipids are available at the outer surface²⁶. As shown in Fig. 2, liposomes having the same phospholipid composition as present in the outer membrane half of either red cell membranes or platelet plasma membranes do not reduce whatever the blank clotting time. On the other hand, liposomes with the same phospholipid composition as the inner half of red cell or platelet plasma membranes produce a threefold reduction of the coagulation time at optimal concentrations. The amount of lipid required is higher than with ghosts, since only a fraction of the total lipid is available at the surface of handshaken liposomes.

It is realised that the measured procoagulant activity of the lipids is not performed at one particular transitory stage of the blood clotting process, in spite of the presence of Russell's viper venom which is known to activate both factor *X* and factor *V* (ref. 27). Nevertheless, the results strongly suggest that the outer surface of blood cells is devoid of phospholipids which are active in blood coagulation, mainly due to the absence of any significant amount of phosphatidylserine. This phospholipid comprises about 30% of the phospholipids at the membrane interior, representing a concentration which has been shown to be optimal in activating prothrombin by the prothrombinase complex¹¹.

The presence of procoagulant phospholipids only at cytoplasmic surfaces of blood cells is not only consistent with phospholipid asymmetry in membranes, but might also represent an important mechanism both in avoiding thrombosis and in regulating haemostasis. It could be judged as potentially dangerous to have procoagulant phospholipids at the outer surface of blood cells, which may bring about a 'permanent' condition of hypercoagulability. This fact itself could provide a physiological reason for an asymmetric phospholipid distribution in blood cell membranes. On the other hand, after disruption of the vessel wall the sequence of platelet adhesion, release reaction and ADP-induced platelet aggregation, presumably accounts for the primary arrest of bleeding. Provided that platelet factor 3 activity becomes accessible during this process, its availability should come after the release reaction since no phosphatidylserine can be labelled from the outside during the release reaction²⁸. Moreover, it has been demonstrated that only a small fraction of lipid procoagulant activity becomes available during release reaction as compared with the activity obtained upon lysis^{29,30}. This raises the question whether during or after formation of the primary haemostatic platelet plug, either (partial) lysis of platelets or an as yet unknown mechanism which translocates phosphatidylserine through the plasma membrane is necessary to provide a catalytic lipid surface for interacting coagulation factors.

R. F. A. ZWAAL
P. COMFURIUS
L. L. M. VAN DEENEN

Laboratory of Biochemistry,
State University of Utrecht,
Transitorium 3,
University Centre 'De Uithof',
Padualaan 8,
Utrecht, The Netherlands

Received 5 April; accepted 30 May 1977.

- ¹ Seegers, W. H., Sakuragawa, N., McCoy, L. E., Sedensky, J. A. & Dombrose, F. A. *Thromb. Res.* **1**, 293–310 (1972).
- ² Papahadjopoulos, D. & Hanahan, D. J. *Biochim. biophys. Acta* **90**, 436–439 (1964).
- ³ Milstone, J. H. *Fedn Proc.* **23**, 742–748 (1964).
- ⁴ Barton, P. G., Jackson, C. M. & Hanahan, D. J. *Nature* **214**, 923–924 (1967).
- ⁵ Hemker, H. C., Esnouf, M. P., Hemker, P. W., Swart, A. C. W. & Macfarlane, R. G. *Nature* **215**, 248–251 (1967).
- ⁶ Jobin, F. & Esnouf, M. P. *Biochem. J.* **102**, 666–674 (1967).
- ⁷ Marcus, A. J. *Adv. Lipid Res.* **4**, 1–37 (1966).
- ⁸ Bangham, A. D. *Nature* **192**, 1197–1198 (1961).
- ⁹ Papahadjopoulos, D., Houghie, C. & Hanahan, D. J. *Proc. Soc. exp. Biol. Med.* **111**, 412–416 (1962).

- ¹⁰ Daemen, F. J. M., van Arkel, C., Hart, H. Ch., van der Drift, C. & van Deenen, L. L. M. *Thromb. Diath. Haemorrh.* **13**, 194–217 (1965).
- ¹¹ Bull, R. K., Jevons, S. & Barton, P. G. *J. biol. Chem.* **247**, 2747–2754 (1972).
- ¹² Gitel, S. N., Owens, W. G., Esmon, C. T. & Jackson, C. M. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1344–1348 (1973).
- ¹³ Bretscher, M. S. *Science* **181**, 622–629 (1973).
- ¹⁴ Zwaal, R. F. A., Roelofs, B. & Colley, C. M. *Biochim. biophys. Acta* **300**, 159–182 (1973).
- ¹⁵ Bretscher, M. S. & Raff, M. C. *Nature* **258**, 43–49 (1975).
- ¹⁶ Marchesi, V. T., Furthmayr, H. & Tomita, M. A. *Rev. Biochem.* **45**, 667–698 (1976).
- ¹⁷ Chap, H. J., Zwaal, R. F. A. & van Deenen, L. L. M. *Biochim. biophys. Acta* **467**, 146–164 (1977).
- ¹⁸ Zwaal, R. F. A., Roelofs, B., Comfurius, P. & van Deenen, L. L. M. *Biochim. biophys. Acta* **406**, 83–96 (1975).
- ¹⁹ Steck, T. L. *Meth. membr. Biol.* **2**, 245–281 (1974).
- ²⁰ Hemker, H. C. *Handbook of Hemophilia* (eds. Brinkhous, K. M. & Hemker, H. C.) 31–48 (Excerpta Medica, Amsterdam, 1975).
- ²¹ Verkleij, A. J. *et al. Biochim. biophys. Acta* **323**, 178–193 (1973).
- ²² Singleton, W. S., Gray, M. S., Brown, M. L. & White, J. L. J. *Am. Oil. Chem. Soc.* **42**, 53–56 (1965).
- ²³ Ansell, G. B. & Spanner, S. *Biochem. J.* **79**, 176–184 (1961).
- ²⁴ Sanders, H. *Biochim. biophys. Acta* **144**, 485–487 (1967).
- ²⁵ Berden, J. A., Barker, R. W. & Radda, G. K. *Biochim. biophys. Acta* **375**, 186–208 (1975).
- ²⁶ Bangham, A. D., de Gier, J. & Greville, G. D. *Chem. Phys. Lipids* **1**, 225–246 (1967).
- ²⁷ Schiffman, S., Theodor, I. & Rapoport, S. I. *Biochemistry* **8**, 1397–1405 (1969).
- ²⁸ Schick, P. K., Kurica, K. B. & Chacko, G. K. *J. clin. Invest.* **57**, 1221–1226 (1976).
- ²⁹ Sixma, J. J. & Nijssen, J. G. *Thromb. Diath. Haemorrh.* **24**, 206–213 (1970).
- ³⁰ Joist, J. H., Dolezel, G., Lloyd, J. V., Kinlough-Rathbone, R. L. & Mustard, J. F. *J. Lab. clin. Med.* **84**, 474–482 (1974).

Ion transport and rotation of bacterial flagella

BACTERIA swim by rotating their flagella^{1–4}. A flagellum consists of a thin helical filament which is joined by a hook to a rod and a set of rings embedded in the cell wall and the plasma membrane^{5,6}. Gram-positive bacteria such as *Bacillus subtilis* have two rings, one of them, the M ring, binding to the plasma membrane and the other, the S ring, being located on the outside of the membrane. Gram-negative bacteria such as *Escherichia coli* have two additional rings which are associated with the peptidoglycan layer and the outer lipopolysaccharide membrane. The energy source of the flagellar motor seems to be not ATP itself but rather an intermediate in oxidative phosphorylation which is interpreted as an electrochemical potential gradient for hydrogen ions across the plasma membrane^{7–9}. Berg^{3,4} has proposed that the rotation of the flagellum is driven by the translocation of ions down an electrochemical gradient. This hypothesis, however, has never been formulated in terms of a specific model. An entirely different mechanism based on hydrodynamic interactions between plasma membrane and basal body of the flagellum has been suggested by Adam¹⁰. Here, we examine Berg's hypothesis more closely and propose a model for the electromechanical coupling between ion flow and flagellar rotation.

Following Berg⁴ we assume that the S ring is attached to the peptidoglycan layer of the cell wall and that the M ring, which is mounted rigidly on the rod, is able to rotate in the plasma membrane (Fig. 1). We further assume that hydrogen ions which have in the extracellular medium a high electrochemical potential with respect to the cytoplasm^{11–13} are able to flow through the interstice between the S and M rings and through the periphery of the M ring to the cytoplasm, as shown in Fig. 1. How can this flow of ions generate a torque?

According to current concepts, the passage of ions through cellular membranes takes place within localised structures termed ion channels. An ion channel may consist of a series of polar ligand groups which partly replace the primary hydration shell surrounding the ion in water. In this way the channel offers to the ion an energetically favourable pathway through the hydrophobic interior of the membrane. A well studied example is the gramicidin channel^{14–16}, which is selective for univalent cations and in which the permeating ion interacts with the carbonyl oxygens of amide groups. In the following we shall assume that the face of the S ring, which is in contact with the M ring, contains radially arranged rows of ligand groups (Fig. 2). If the opposite contact face of the M ring contains similar rows of ligand groups, but arranged in a non-radial direction, the two sets of rows intersect at a certain angle. Evidence for a ring structure with non-radial arrangement

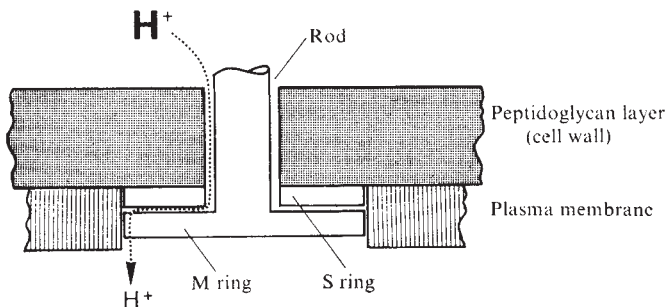


Fig. 1 Structure of the basal body of the flagellum of Gram-positive bacteria, according to De Pamphilis and Adler⁵ and to Berg⁴. The S ring is attached to the cell wall and the M ring, which is mounted rigidly on the rod, is able to rotate in the plasma membrane. Hydrogen ions are assumed to flow down an electrochemical gradient from the outside through the interstice between S and M ring and through the periphery of the M ring to the cytoplasm.

of subunits has been obtained by De Pamphilis and Adler⁵ in their electron microscopic studies of bacterial flagella, although it was not possible to assign the observed non-radial ring structure to one specific type of ring (M, S, P or L).

Figure 2 shows that, when the S ring is held fixed and the M ring is rotated, the points of intersection move radially with respect to the S ring. We further assumed that the energy of interaction of the ion with one ligand row is not sufficient to compensate for the energy required to remove the ion from water but that an energetically favourable state is reached if the ion is located at the site of intersection where it has twice as many ligand groups available. In these circumstances the ion is constrained to move together with the site of intersection. In the model depicted in Figs 1 and 2, part of the electrochemical gradient of H^+ drops across the rings in a radial direction so that an ion located between the S and M rings experiences a radial (centrifugal) force. As we have assumed that ions are confined to the intersection sites between the two sets of rows, an ion movement down the radial potential gradient results in a rotation of the M ring with respect to the S ring. In this way, ions entering the ring system near the axis and leaving it at the periphery, drive the rotation of the flagellum in a manner not unlike the action of a turbine.

If K is the radial force acting on the ion and θ the angle between the two intersecting ligand rows (Fig. 2), the torque exerted by an ion on the M ring is equal to $(a/2)K \sin 2\theta$, where a is the distance of the intersection point to the centre. The difference in electrochemical potential of H^+ between cytoplasm and extracellular space may be expressed as $e_0 \Delta\psi_{eq}$ where e_0 is the charge of the proton and $\Delta\psi_{eq} = \Delta\psi - (59 \text{ mV}) \Delta pH$ is the equivalent electrical potential difference ($\Delta\psi$ is the membrane potential). If the fraction α of $\Delta\psi_{eq}$ acts across the width ($R_2 - R_1$) of the rings (R_1 and R_2 are the inner and the outer diameter of the rings, respectively) and if the turbine contains on the average n hydrogen ions, then the mean torque M (averaged over one repetition period) is given by

$$M = \alpha B n e_0 \Delta\psi_{eq}$$

where B is a dimensionless quantity which depends on the geometry of the ligand system. It may be shown that for the model represented in Fig. 2 the relation $B = \rho \sin^2 \psi / (\phi - \arcsin(\rho \sin \phi))$ holds, where $\rho = R_1/R_2$ and ϕ is the tilt angle of the non-radial ligand row (Fig. 2). From the structural data of the basal body⁵ a value of $\rho \approx 0.3$ is obtained. If the tilt angle is chosen to be $\phi = 60^\circ$, one finds that $B \approx 0.3$. Numerous attempts have been made to determine the electrochemical gradient for H^+ across the bacterial plasma membrane, and there is now considerable evidence that in respiring bacteria $\Delta\psi_{eq}$ is of the order of 0.1–0.2 V (cytoplasmic side nega-

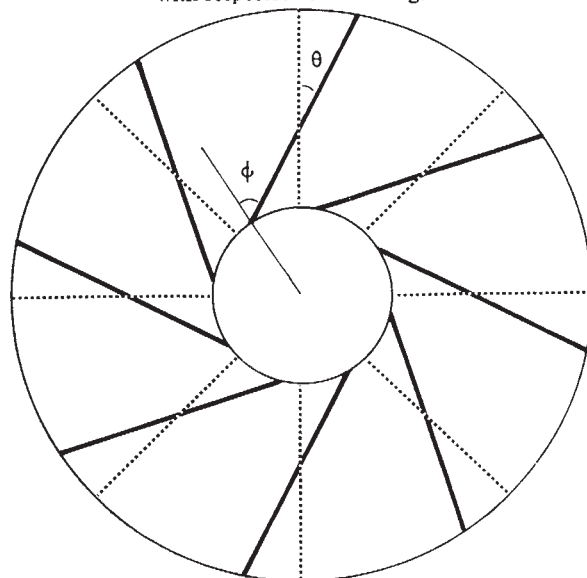
tive)^{11–13}. Using these values of $\Delta\psi_{eq}$ and B , and assuming, for the purpose of an estimate to the order of magnitude, $\alpha \approx 1$ and $n \approx 10$, one obtains a torque of $M \approx 10^{-19} \text{ J}$. This figure may be compared with the actual torque generated by the flagellar motor. From a hydrodynamic analysis of flagellar motion, Coakley and Holwill¹⁷ estimated that the power dissipation of a single flagellum rotating at a frequency of 50 Hz is about $P = 4 \times 10^{-17} \text{ W}$. This corresponds to a torque $M = P/\Omega$ of $1.3 \times 10^{-19} \text{ J}$ (Ω is the angular velocity). Similar values of M were obtained from experiments with tethered bacteria described by Silverman and Simon² and by Berg³. Thus, the mechanism considered above is able to generate a torque of the required order of magnitude. The predicted torque M is independent of angular velocity Ω and the external load which means that $\Omega = M/f$ should decrease when the friction coefficient f is increased. This is consistent with the experimental findings of Berg³ who observed that the rotational frequency of tethered cells was approximately inversely proportional to the viscosity of the external medium (see also Schneider and Doetsch²⁴).

The number of ions flowing through the turbine in one second may be estimated to be $P/e_0 \Delta\psi_{eq} \approx 4 \times 10^{-17} / 1.6 \times 10^{-19} \times 0.2 \approx 10^3$ (an estimate of 10^3 ions per cycle has been given by Berg³ for a tethered cell spinning at 10 Hz). If the number v of ligand rows on each ring is assumed to be identical with the number of subunits found in the electron microscopic study of De Pamphilis and Adler⁵ ($v = 16$), the rate of ion flow along one row is of the order of 100 s^{-1} . If a single row contains about 10 ion-binding sites then the required jump rate of the ion between adjacent sites is $\sim 10^3 \text{ s}^{-1}$. This value is well within the range of feasible jump rates in ion channels¹⁸.

In recent years, it has become clear that bacterial chemotaxis is based on the ability of bacteria to reverse the direction of rotation of their flagella in response to sensory stimuli^{19,20}. A reversal of the direction of rotation could occur by a conformational transition of the subunits of the M ring¹⁰, which would change the tilt angle of the ligand rows from ϕ to $-\phi$ (Fig. 2). Such a conformational transition could be triggered by a transient change in membrane potential^{21–23}.

Several modifications of the proposed model are conceivable. Other ion species besides H^+ which have a non-equilibrium distribution across the cytoplasmic membrane may drive the flagellar motor. Furthermore, the geometry of the ligand rows depicted in Fig. 2 is certainly not optimal and many other

Fig. 2 Hypothetical arrangement of ligand groups on the S and M rings. Ligand rows on the S (dashed) and M rings (full lines) are superimposed. When the M ring is rotated the points of intersection, which are assumed to be ion-binding sites, move radially with respect to the S ring.



arrangements are feasible. Another (less likely) alternative would consist in assuming that the torque is generated between the rod and the bushing in the cell wall, the rod being equipped with straight axial and the bush with helical ligand rows (or vice versa).

I thank Winfried Boos and Gerold Adam for interesting discussions.

P. LÄUGER

Department of Biology,
University of Konstanz,
D-775 Konstanz, FRG

Received 4 March; accepted 8 June 1977.

- ¹ Berg, H. C. & Anderson, R. A. *Nature* **245**, 380–382 (1973).
- ² Silverman, M. & Simon, M. *Nature* **249**, 73–74 (1974).
- ³ Berg, H. C. *Nature* **249**, 77–79 (1974).
- ⁴ Berg, H. C. *Nature* **254**, 389–392 (1975).
- ⁵ De Pamphilis, M. L. & Adler, J. *J. Bact.* **105**, 384–395 (1971).
- ⁶ De Pamphilis, M. L. & Adler, J. *J. Bact.* **105**, 396–407 (1971).
- ⁷ Larsen, S. H., Adler, J., Gargus, J. J. & Hogg, R. W. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1239–1243 (1974).
- ⁸ Thirayathasana, P. & Valentine, R. C. *Biochim. biophys. Acta* **347**, 464–468 (1974).
- ⁹ Ordal, G. W. & Goldman, D. J. *J. molec. Biol.* **100**, 103–108 (1976).
- ¹⁰ Adam, G. *J. theor. Biol.* **65**, 713–726 (1977).
- ¹¹ Altendorf, K., Hirata, H. & Harold, F. M. *J. biol. Chem.* **250**, 1405–1412 (1975).
- ¹² Ramos, S., Schuldiner, S. & Kaback, H. R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1892–1896 (1976).
- ¹³ Padan, E., Zilberstein, D. & Rottenberg, H. *Eur. J. Biochem.* **63**, 533–541 (1976).
- ¹⁴ Urry, D. W. *Proc. natn. Acad. Sci. U.S.A.* **68**, 672–676 (1971).
- ¹⁵ Hladky, S. B. & Haydon, D. A. *Biochim. biophys. Acta* **274**, 294–312 (1972).
- ¹⁶ Bamberg, E. & Läuger, P. *J. membr. Biol.* **11**, 177–194 (1973).
- ¹⁷ Coakley, C. J. & Holwill, M. E. *J. theor. Biol.* **35**, 525–542 (1972).
- ¹⁸ Läuger, P. *Biochim. biophys. Acta* **311**, 423–441 (1973).
- ¹⁹ Adler, J. A. *Rev. Biochem.* **44**, 341–356 (1975).
- ²⁰ Berg, H. C. *A. Rev. Biophys. Bioengng.* **4**, 119–136 (1975).
- ²¹ Caraway, B. H., Krieg, N. R. *Can. J. Microbiol.* **18**, 1749–1759 (1972).
- ²² Faust, M. A. & Doetsch, R. N. *Can. J. Microbiol.* **17**, 183–189 (1971).
- ²³ Szmecman, S. & Adler, J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4387–4391 (1976).
- ²⁴ Schneider, W. R. & Doetsch, R. N. *J. Bact.* **117**, 696–701 (1974).

Analysis of positional information applied to cirral patterns of the ciliate *Euplotes*

IN studying the relative positions of structural features on the surfaces of organisms, most biologists are content with qualitative impressions of pattern. This approach is inadequate when there are large variations among individuals, as occurs in the taxonomically important positional patterns formed by the locomotory organelles, the cirri, of the hypotrich ciliate genus *Euplotes*. The frequency distribution of all intercirral distances provides a quantitative measure of cirral pattern that incorporates individual variations. A comparative study¹ among different *Euplotes* populations of such distributions reveals a small set of basic cirral patterns. It also demonstrates that certain cirral patterns may be simulated by morphogenetically plausible alterations in others, thus supporting the hypothesis that certain cirral patterns are derivative of others. The theoretical creation of artificial positional patterns that mimic naturally occurring patterns provides a useful perspective on the systematics of this group of organisms.

The ventral surface of *Euplotes* (Fig. 1) bears a system of morphogenetically unified landmarks, the frontoventral and transverse cirri, which arise during asexual division from subpellicular kinetosomal plaques. These compound ciliary organelles develop from the simultaneous elongation and condensation of a series of five anteroposterior kinetosomal streaks². The posterior portions give rise to the five transverse cirri found universally throughout the genus. Anteriorly, at most two additional cirri develop from each streak, forming the frontoventral cirri. It is the number and relative positions of cirri along the streaks that determine the cirral pattern. In most of the 51 recognised species³, there are 10 frontoventral cirri, as in *E. harpa* (Fig. 1), with a 'cirrotype' of 10. But some species are of lesser cirrotype, with several common freshwater forms being of cirrotype 9, and a few marine species of cirrotypes 8 and 7.

A simple measure of cirral pattern is obtained by calculating, for each specimen of a sample, from coordinate data for the

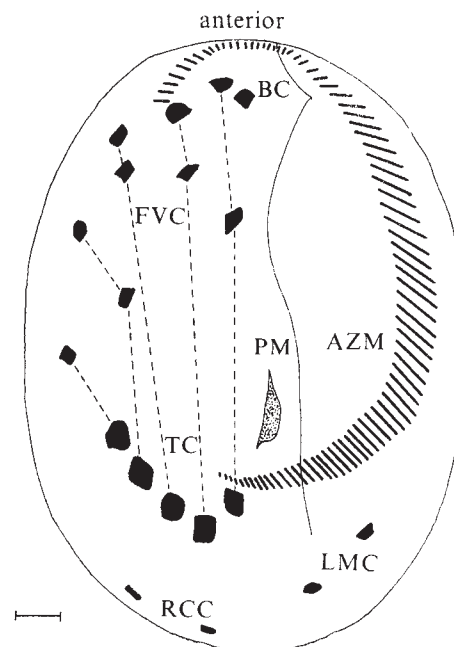


Fig. 1 Schematic diagram of the ventral surface of a silver-stained specimen of *E. harpa*, a brackish-water species with cirrotype 10, illustrating the basal plaques of the frontoventral (FVC) and transverse (TC) cirri as well as the longitudinal subpellicular streaks (dashed lines) from which these cirri arise during morphogenesis. Also illustrated are the buccal cirrus (BC), which is treated as part of the preceding system; the adoral zone of compound ciliary membranelles (AZM) that line the mouth; the right caudal cirri (RCC); the left marginal cirri (LMC), and the paroral membrane (PM). The line scale indicates 10 μ m.

positions of the frontoventral and transverse cirri (including the buccal cirrus), all intercirral distances. These distances are scaled by a factor external to the cirral system proper, the length of the specimen. For any given population ($n \geq 25$) of *Euplotes*, regardless of cirrotype or mean size, the frequency distribution of such relative intercirral distances provides a measure of the cirral pattern of that population. The distributional profiles of different populations then may be compared using the maximum difference (D_{max}) between their cumulative frequency distributions, that is, the Kolmogorov-Smirnov statistic⁴.

To assess the extent of inherent variability in cirral patterns, 17 subclonal populations of the largest species in the genus, the brackish-water form *E. harpa*, were grown in different conditions of temperature and salinity during 3 yr. Samples of at least 50 specimens were selected from each population. All specimens were selected randomly from preparations silver-stained by the Chatton-Lwoff procedure⁵, except that only non-reorganising, well impregnated and properly oriented specimens in micronuclear interphase⁶ were chosen. Figure 2 shows the relative frequency distributions for the two most dissimilar samples from these populations, all of which were derived from the same original individual. For these two distributions, D_{max} is 0.0693, and this value provides a conservative criterion for judging the similarities of other distributions. If two samples of, for example, different species differ in their distributions by less than the difference that can exist among subclones of *E. harpa*, it would be unwise to consider their cirral patterns to be different.

By this criterion, many of the numerous species of *Euplotes* have indistinguishable cirral patterns¹. Most cirrotype 10 patterns are very similar, with only two of 13 geographically disparate samples differing slightly from the common pattern (Fig. 1). Indeed, for each cirrotype known to exist within the

genus, at most two cirral patterns exist, as judged by comparisons of the distributions of 33 clonal samples of diverse origin. The internal integrity of each of these few patterns permits the following interpretation of cirral pattern diversity within the genus.

There are only two ways in which a *Euplotes* may possess a cirrotype of 9. The first, as in *E. patella*, involves the absence of a cirrus at the position in which the third cirrus (numbering from posterior to anterior) in the fourth streak (numbering from the organism's left to right), cirrus 3/IV, occurs in *Euplotes* of cirrotype 10. The second variant, as in *E. affinis*, occurs as a result of the absence of the 2/IV cirral position. That is, there are only two cirri in streak IV in forms of cirrotype 9. In the more common variant, the second cirrus occurs at the anterior end of the streak, while in the rarer variant, it occurs in the middle. Results of a distributional analysis based on 16 samples confirm the existence of these two distinct patterns⁷ and indicate that the latter variant is the more homogeneous cirral pattern; nonetheless, the more common pattern is not more variable than the subclones of *E. harpa*.

The integrity of each of the above cirral patterns suggests that the cirral patterns of forms with cirrotypes less than 10 can be explained by a simple loss of cirri (either by the direct depletion of kinetosomal numbers in the affected streaks or by the redistribution during morphogenesis of unchanged numbers of kinetosomes). To test this hypothesis, several artificial distributions were constructed by the recalculation of intercirral distance distributions after the removal of the coordinate data for appropriate cirri from samples of *Euplotes* of cirrotype 10. Then these were compared with the distributions of forms with cirral 'deficiencies' presumed to be homologous with the artificial forms.

Figure 3 illustrates the distributions for two freshwater samples of *Euplotes* of cirrotype 9 with the *affinis*-type cirral pattern and the artificial distribution obtained subsequent to the removal of cirrus 2/IV from a freshwater sample of *Euplotes* of cirrotype 10. The fit is remarkably good ($D_{\max} = 0.0424$) and well within the range of variation of subclones of *E. harpa*. An adequate fit ($D_{\max} = 0.0612$) to a *patella*-type cirral pattern

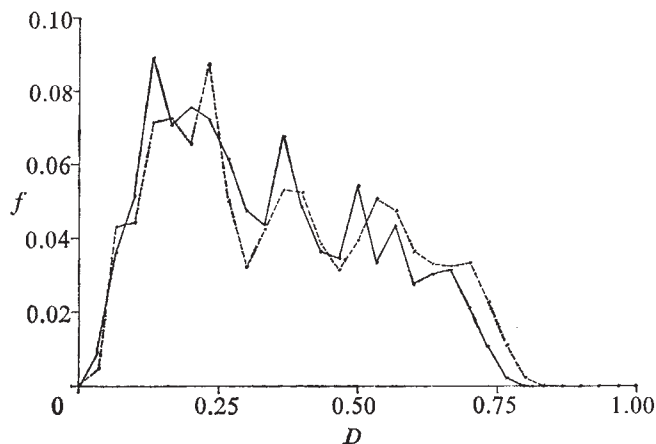


Fig. 2 Relative frequency (f) distributions of scaled intercirral distances (D) for the two most dissimilar ($D_{\max} = 0.0693$) of 17 *E. harpa* subclones, based on samples of 57 (dashed line) and 55 (solid line) specimens. Coordinate data for each specimen were obtained by placing a Leitz Ortholux microscope with drawing tube on top of an electronic two-dimensional digitiser. Data were recorded on cassette tape, transferred to nine-track magnetic tape, and thence to on-line disk storage for processing on an IBM 370-165-II computer system, using a Fortran program written by me. For each of the 2,122 specimens, the coordinate data were visually checked by inspection of computer plots. Intercirral distances and their distributions were calculated from the coordinate data, Gould plots were made, and comparisons among distributions were performed automatically.

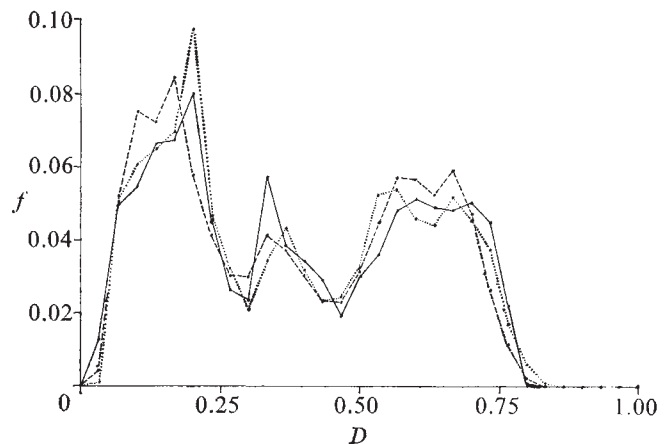


Fig. 3 Relative frequency distributions for two samples of *Euplotes* with cirrotype 8: *E. affinis* from Stevenage, Hertfordshire, UK (dashed line), and from Toronto, Ontario, Canada (dotted line); and an artificial *affinis*-type sample (solid line) created from a sample of *Euplotes* with cirrotype 10 from Inuvik, Northwest Territories, Canada, by the removal of the coordinate data for two cirri.

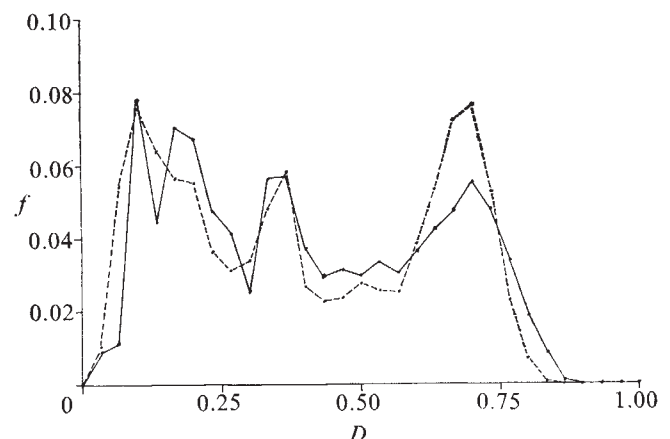
was also obtained from this sample after the deletion of cirrus 3/IV. Similarly, reasonable simulations of both cirrotype 8 and both cirrotype 7 patterns were obtained using artificial distributions derived from the small marine species *E. minuta* (Fig. 4).

These results demonstrate the plausibility of explaining certain cirral patterns as derivative from others. In conjunction with quantitative studies of variation in other taxonomic features⁸, it is possible to suggest a phylogeny for the genus *Euplotes* that eschews untested typological dichotomies¹.

The analysis of positional data by means of the distributions of interpositional distance is an approach to biological pattern analysis that has obvious applications. Not only may it be applied to the systematics of other hypotrich genera, in which cirri exist in fixed patterns, and to, for example, setal positions in various insects, but it also may be used to study the changes in positional relationships that occur during development in various taxa. It may be possible to show, for example, that certain patterns are arrested stages in the development of others, as is undoubtedly the case with some of the oxytrichid hypotrichs⁹ among the ciliated protozoa.

This work was financed by grants from the National Research

Fig. 4 Relative frequency distributions for an *E. raikovi* sample (dashed line) from Rye Harbor, New Hampshire, and an artificial sample with cirrotype 7 (solid line) created from a sample with cirrotype 10, *E. minuta*, from Villefranche-sur-Mer, France, by removal of the coordinate data for 2/IV, 2/II and 2/I cirri ($D_{\max} = 0.0618$).



Council of Canada to myself and J. Berger, whom I thank for advice and criticism. I thank M. E. Park for a critical reading of this report.

MICHAEL A. GATES*

Department of Zoology,
University of Toronto,
Toronto, Ontario M5S 1A1, Canada

Received 5 May; accepted 16 June 1977.

*Present address: Culture Centre of Algae and Protozoa, 36 Storey's Way, Cambridge, UK.

¹ Gates, M. A. thesis, Univ. Toronto (1976).

² Ruffolo, J. J. *J. Morph.* **148**, 489–528 (1976).

³ Curds, C. R. *Bull. Br. Mus. nat. Hist. (Zool.)* **28**, 1–61 (1975).

⁴ Sokal, R. R. & Rohlf, F. J. *Biometry* 571–575 (Freeman, San Francisco, 1969).

⁵ Corliss, J. O. *Stain Tech.* **28**, 97–100 (1953).

⁶ Prescott, D. M., Kimball, R. F. & Carrier, R. F. *J. Cell Biol.* **13**, 175–176 (1962).

⁷ Washburn, E. S. & Borror, A. C. *J. Protozool.* **19**, 604–608 (1972).

⁸ Frankel, J. J. *Embryol. exp. Morph.* **33**, 553–580 (1975).

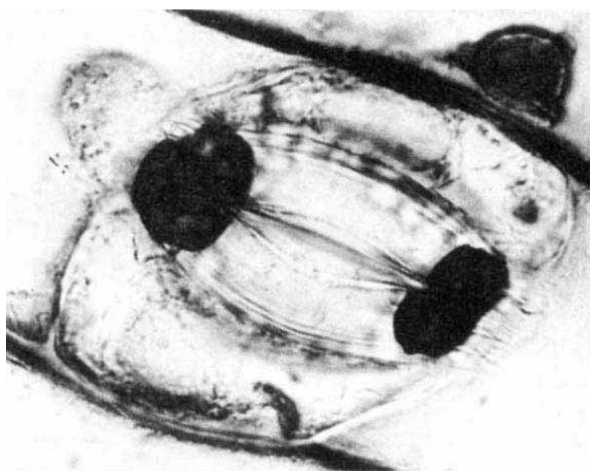
⁹ Borror, A. C. *J. Protozool.* **19**, 1–23 (1972).

Ion-adsorbent substomatal structures in *Tradescantia pallidus*

STOMATAL movements result from turgor imbalances occurring within the stomatal complex which are largely brought about by differential potassium distribution between the guard cells and adjacent epidermal cells^{1–4}. The guard cell vacuole has been established as the sink of the potassium flux during stomatal opening^{5,6}, but the source of the ions has not been identified. It is generally believed that the ion flux is apoplastic because plasmodesmata have only occasionally been observed between the guard and adjacent epidermal cells^{7–9}. We have described substomatal ion-adsorbent sites in some species of filicoid ferns and Commelinaceae which we believe could act as the immediate sink/source of potassium ions involved in stomatal closing and opening¹¹. Discrete accumulations of Macallum's stain for potassium¹² were demonstrated at both poles of the guard cell complex on the face adjacent to the mesophyll. These sites corresponded to substomatal endocuticular swellings observed by scanning electron microscopy which, when collapsed, revealed the presence of a disk-shaped structure on the outer face of the guard cell walls at the site of ion adsorption. Direct observation of the ion-adsorbent sites was prevented by the overlying endocuticular substomatal sacs. We have now observed these sites directly in *Tradescantia pallidus* and obtained evidence which strongly implicates them in stomatal functioning.

Tradescantia pallidus was selected as a more suitable material for further investigations into the form and function of substomatal ion-adsorbent sites because its stomata respond readily

Fig. 1 Stomatal complex of *Tradescantia pallidus* stained for potassium showing the polar accumulations ($\times 1,000$).



to physiological experimentation and it has well developed ion-adsorbent sites, as determined by the Macallum technique¹¹ (Fig. 1). Segments of fresh whole leaves were mounted lengthwise on a scanning electron microscope stub and cut longitudinally, along the longitudinal axes of the stomata, immediately before being plunged into liquid nitrogen. The specimen was then mounted and observed on the cryostage of a JSM-35 scanning electron microscope in an uncoated condition.

Our findings indicate that the ion-adsorbent sites in this species consist of discrete subtetrahedral bodies lying in the intercellular space between the pole of the guard cell and the adjacent polar subsidiary cell (Fig. 2a). The ion-adsorbent bodies are closely paired so that each pole of each guard cell is associated with its own single body. While one face of the ion-adsorbent body is addressed onto the subsidiary cell wall, there seem to be no direct connections between the body and the protoplasts of either the guard or subsidiary cells. Each body has an approximate volume of $0.28 \times 10^{-15} \text{ m}^3$ in comparison

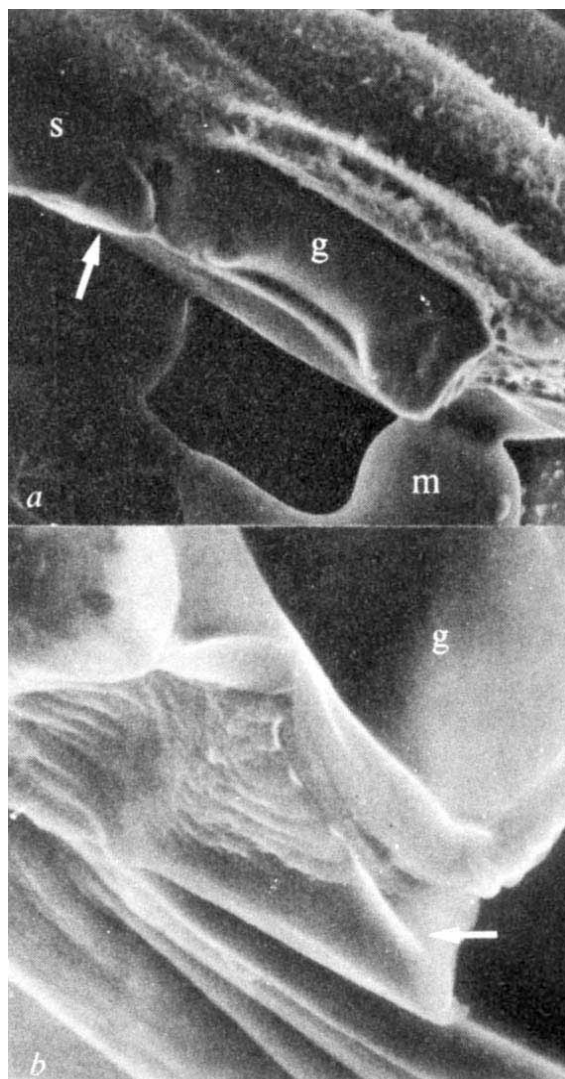


Fig. 2 a, Scanning electron micrograph of a vertical longitudinal section through the lower epidermis of *Tradescantia pallidus*. The section passed exactly through the centre of a stomatal complex leaving a single guard cell (g) and polar subsidiary cell (s). The mesophyll tissue (m) indicates the orientation of the section. An ion-adsorbent body (arrowed) can be seen in the intercellular space between the pole of the guard cell and the adjacent subsidiary cell ($\times 1,000$). b, Scanning electron micrograph of a vertical section through the polar region of a guard cell (g) of *Tradescantia pallidus*. The internal paradermal wall of the guard cell has laminate structure with free spaces (arrowed) lying between successive layers ($\times 5,000$).



Fig. 3 Preliminary electron microprobe analyses of the stomatal complex of *Tradescantia pallidus*. The probe was about 6 μm in diameter at an accelerating voltage of 18 keV for 100 s. The peak to the immediate right of the potassium peak in the spectra is of a composite nature in that it is made up of potassium K_{β} and calcium K_{α} emissions. *a*, K_{α} spectral emission from the ion-adsorbent body illustrated in Fig. 2*a*; *b*, K_{α} spectral emission from the polar region of the adjacent guard cell illustrated in Fig. 2*a*.

with the guard cell lumen volume of about $7.85 \times 10^{-15} \text{ m}^3$; the combined volumes of a pair of ion-adsorbent bodies associated with a single guard cell, is, therefore, in the order of 7% of the guard cell volume.

When viewed from the side adjacent to the mesophyll, the bodies can be seen to be covered by the endocuticle, which is strongly plicated at the pole of the guard cell and over the immediately adjacent areas of the polar subsidiary cells, the bodies are similar to the trabeculae reported on from the stomata of *Polypodium vulgare*¹⁰. Sections through the polar region of the guard cells indicate that the extracellular space, in which the ion-adsorbent bodies are located, is contiguous with spaces present in the matrix of the lower paradermal wall of the guard cell (Fig. 2*b*). These spaces are not apparent in thin sections observed under the transmission electron microscope (probably as a result of fixation procedures), although the differentially-thickened cell wall in this region seems to be strongly laminate.

Electron microprobe analysis of the ion-adsorbent body (Fig. 3*a*) suggests that it is particularly rich in potassium compared with the protoplast at the pole of the adjoining guard cell (Fig. 3*b*). In addition, the body also contains appreciable quantities of silicon, sulphur, phosphorus and chlorine, while only the latter two are detectable in comparable amounts in the guard cell protoplast.

Our findings conform to the hypothetical arrangement proposed for the substomatal structures as found in *Polypodium vulgare*¹⁰. The ion-adsorbent bodies are situated in an extracellular position in both species studied, although their exact location differs. The bodies are in close association with the outer

face of the lower paradermal cell walls at the poles of the guard cell complex in *Polypodium vulgare*, while in *Tradescantia pallidus* they are situated in the intercellular space between the pole of the guard cell complex and the polar subsidiary cells.

Potassium is the most important cation involved in the stomatal mechanism and the considerable concentrations of the ion detected in the ion-adsorbent bodies must strongly implicate their involvement in stomatal movements. Being situated as they are at the end of the epidermal water pathway¹², the bodies are in a strategic position to adsorb potassium, among other ions, from the transpiration stream. The bodies can thus have a dual role. They maintain a store of bound potassium which can be released, as and when required, for incorporation into the guard cell vacuole. At the same time, they remove other potentially toxic ions from the transpiration stream (our unpublished data) which could otherwise become lodged elsewhere in the apoplast, and possibly be taken up in the symplast, as water leaves the tissues at the evaporative sites around the stomata.

The scanning electron microscopy was carried out at JEOL (UK) Ltd, London, and we thank Mr E. Toulson for technical assistance.

R. A. STEVENS
E. S. MARTIN

School of Environmental Sciences,
Plymouth Polytechnic,
Drake Lines,
Plymouth, UK

Received 19 April; accepted 31 May 1977.

- 1 Fischer, R. A. & Hsiao, T. C. *Plant Physiol.* **43**, 1953–1958 (1968).
- 2 Sawhney, B. L. & Zelitch, I. *Plant Physiol.* **44**, 1350–1354 (1969).
- 3 Humble, G. D. & Raschke, K. *Plant Physiol.* **48**, 447–453 (1971).
- 4 Raschke, K. in *Transport and Transfer Processes in Plants* (eds Wardlaw, I. F. & Passioura, J. B.) ch. 19 (Academic, New York, San Francisco, London, 1976).
- 5 Heller, F. O., Mausch, W. & Trapp, L. *Naturwissenschaften* **58**, 419 (1971).
- 6 Penny, M. G. & Bowling, D. F. *Planta* **119**, 17–25 (1974).
- 7 Litz, R. E. & Kimmins, W. C. *Can. J. Bot.* **46**, 1603–1605 (1968).
- 8 Fujino, M. & Jinno, M. *Sci. Bull. Fac. Educ., Nagasaki Univ.* **23**, 101–111 (1972).
- 9 Pallas, J. E., Jr & Mollenhauser, H. H. *Am. J. Bot.* **59**, 504–514 (1972).
- 10 Stevens, R. A. & Martin, E. S. *Nature* **265**, 331–334 (1977).
- 11 Macallum, A. B. *J. Physiol., Lond.* **32**, 95–128 (1905).
- 12 Meidner, H. *J. exp. Bot.* **26**, 666–673 (1975).

Heterogeneous population of mitochondrial DNA molecules in higher plants

THE physicochemical properties of mitochondrial (mt)-DNA of higher plants have been reported^{1–4}. mt-DNAs occur as closed circles with molecular weights of $(60–70) \times 10^6$ and it is likely that each mitochondria contains several of these circles. mt-DNAs isolated from different higher plants exhibit similar physicochemical parameters: all mt-DNAs so far analysed^{1–5} in higher plants band at a unique buoyant density of 1.706 g cm^{-3} in neutral CsCl gradients and the few published melting points and reassociation kinetics are not significantly different^{2–4}. Not enough values have been reported to state on possible differences in the contour length measured by electron microscopy. Thus, mt-DNAs isolated from different higher plants cannot be specifically identified by conventional physicochemical techniques. We have recently reported that the analysis of restriction nuclease digests by gel electrophoresis provides a very sensitive test to identify specifically chloroplastic (cp)-DNAs isolated from different plant genera and species⁶. We show here that this technique also allows the characterisation and the specific identification of mt-DNAs isolated from various higher plants. We used *EcoRI* specific cleavage to evidence the general occurrence of heterogeneous population of mt-DNA molecules within an higher plant and to detect clear differences between mt-DNAs extracted from normal and cytoplasmic male-sterile wheat.

Etiolated seedlings of *Cucumis sativus* L. var. Vert long maraicher, *Triticum aestivum* var. Capitole, *T. aestivum* var. Florence Aurore and *T. aestivum* var. Gaines (normal and male sterile lines) were used. *Solanum tuberosum* var. Bintje was grown as callus tissue⁷. Liquid cell suspensions of normal and

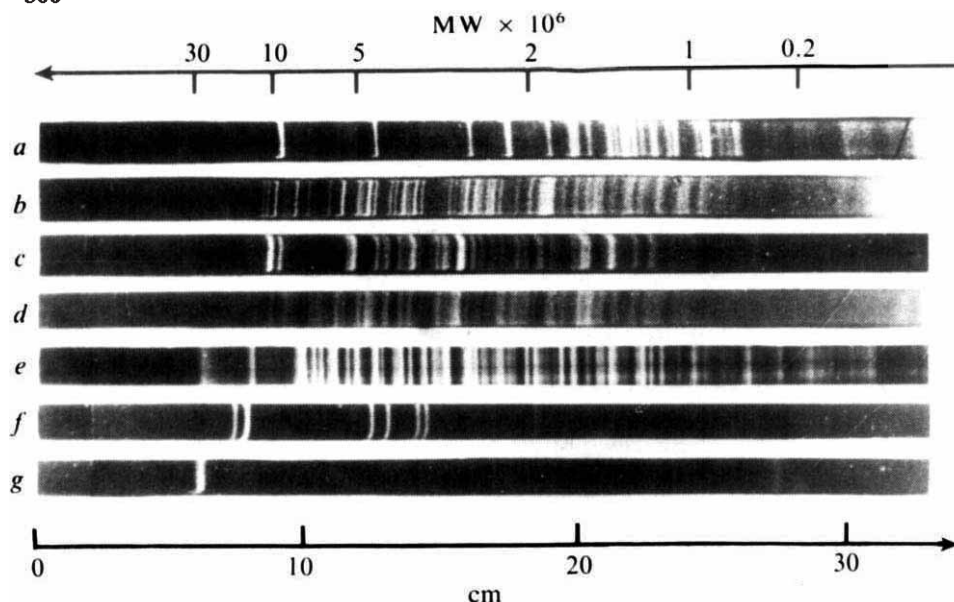


Fig. 1 Agarose slab gel electrophoresis of *Eco*RI digests of mt-DNAs from: *b*, wheat (*T. aestivum* var. Capitole); *c*, potato; *d*, cucumber; *e*, Virginia creeper. Wheat (*T. aestivum* var. Capitole) cp-DNA (*a*) and λ plac5 DNA (*f*) were used for comparison and molecular weight standards respectively. The arrow indicates the direction of migration. Homogenisation of plantlets (etiolated, 10-cm long, roots removed) was carried out with a Waring blender (3 $\times$ 5 s, maximum speed); calluses and liquid cultured cells were disrupted by a French press (3,000 p.s.i.) in buffer A (ref. 10). The homogenate was filtered through a 40- μ m nylon net, centrifuged at 1,200g for 10 min and the supernatant spun at 15,000g for 15 min. The pellet was dispersed with a Potter homogeniser, recentrifuged at 1,200g and 15,000g, and dispersed again. A DNase treatment was achieved at 50 μ g cm $^{-3}$ (DNase I), 4 $^{\circ}$ C for 1 h and the DNase was removed by 2 washings in buffer B (ref. 10) at 15,000g for 15 min. The dispersed pellet was loaded on a 30–60% sucrose gradient in buffer B; after 1 h at 22,000 r.p.m. in an SW.25 II rotor, the mitochondrial band was recovered, diluted with buffer B and pelleted. DNA was isolated as described⁶ for cp-DNA. The supercoiled DNA band was diluted with buffer C (ref. 10) and run at 35,000 r.p.m. overnight in an SW41 rotor. The pellet was suspended in 0.1 M NaCl, 10 $^{-2}$ M Tris, 10 $^{-3}$ M EDTA, pH 8 and the liquid was cleared by a 30-s centrifugation at 20,000g (Eppendorf microfuge); the drug was removed by chromatography on a Dowex 50 Na $^{+}$ column (20 $\times$ 4 mm). Purity and circularity were checked as for cp-DNA (ref. 6). *Eco*RI was purchased from Miles or prepared according to ref. 11. Experimental conditions for digestion, electrophoresis and ultraviolet fluorescence photography were as before⁶, except that the 0.7% Agarose electrophoresis was achieved on 38-cm long, 15-cm wide (eight slots), 4-mm thick slab gels.

tumorous *Parthenocissus tricuspidata* L. were cultured in darkness⁸ and collected during the exponential growth phase. Mitochondria were purified by two cycles of differential centrifugations and cleared from contaminating DNA by a DNase treatment. Mitochondria were freed from DNase by two washes and further purified by a sucrose gradient. The supercoiled mt-DNAs were isolated as the lower ultraviolet fluorescent band of propidium diiodide or ethidium bromide CsCl gradients⁹ and made drug-free by a short Dowex chromatography. The purity was checked by analytical CsCl gradients (unimodal and sharp band at 1.706 g cm $^{-3}$) and the circularity was controlled by a Kleinschmidt spreading followed by positive staining with acid-alcoholic uranyl acetate⁹. The purified mt-DNAs were then digested by saturating amounts of *Eco*RI, *Bam*I or *Sal*I, and the digests analysed by electrophoresis on 38-cm long, 0.7% Agarose slab gels. *Eco*RI restriction fragments given by λ plac 5 DNA and by wheat cp-DNA were used as molecular

weight standards. Gels were stained with ethidium bromide and ultraviolet fluorescence photographs were taken.

Figure 1 shows the electrophoretic pattern of the mt-DNAs extracted from cucumber, potato, wheat and Virginia creeper. Some 50 distinct bands were resolved in each case, about 40 moved more slowly than the bromophenol blue. Like cp-DNA, the mt-DNA of a given plant seems to be characterised by a specific digestion pattern and may be easily distinguished from that of another plant belonging to a different genus. These patterns were found easily reproducible: several independent preparations of each mt-DNA were individually digested and lead to rigorously reproducible banding patterns, whatever the digestion time (3–12 h). The same number of bands was obtained on 58-cm Agarose gels in individual tubes.

mt-DNA isolated from three hexaploid wheats, *Triticum aestivum* var. Capitole, *T. aestivum* var. Florence Aurore and *T. aestivum* var. Gaines display an identical pattern (Fig. 2,

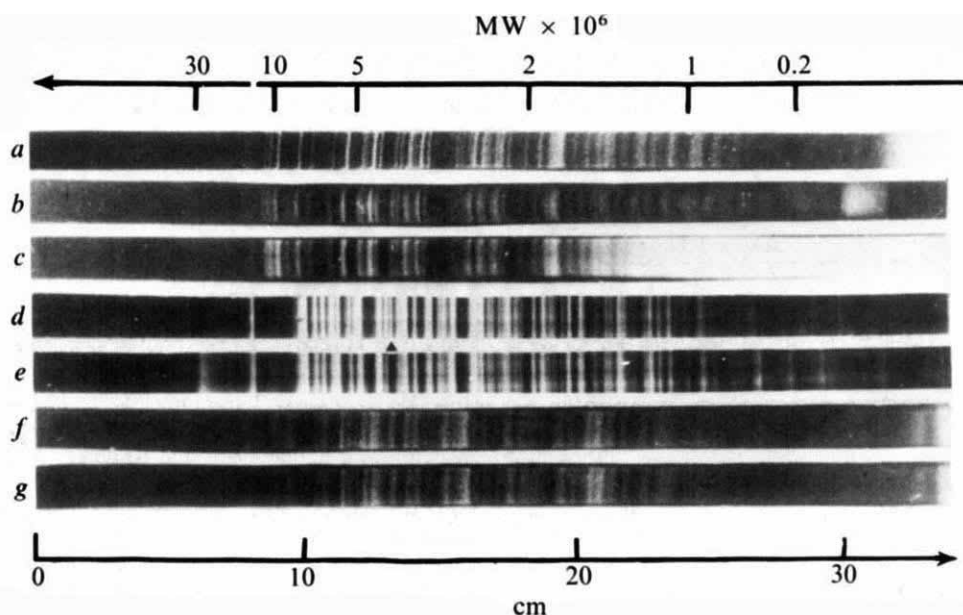
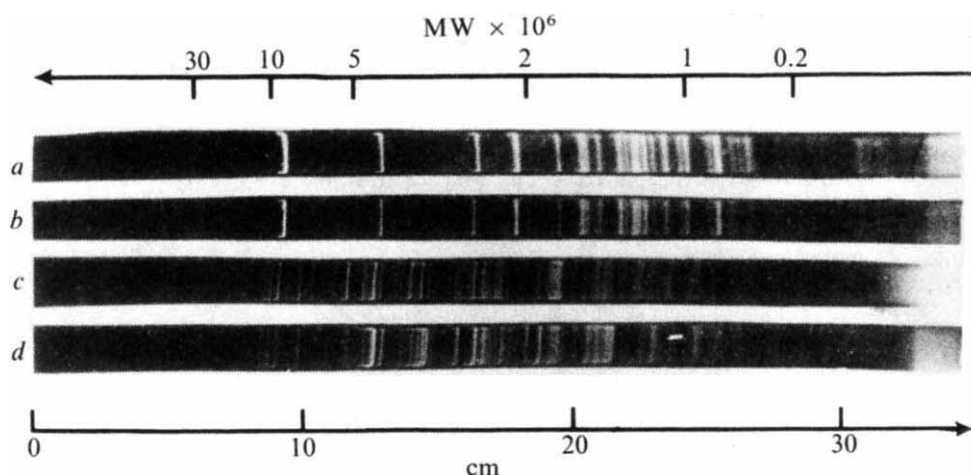


Fig. 2 Agarose gel electrophoresis of *Eco*RI digests of mt-DNAs isolated from: *a*, *Triticum aestivum* var. Capitole; *b*, *T. aestivum* var. Florence Aurore; *c*, *T. aestivum* var. Gaines; *d*, liquid cultured normal cells of *Parthenocissus tricuspidata* L.; *e*, liquid cultured crown-gall cells of *P. tricuspidata* L.; *f*, cucumber hypocotyls; *g*, cucumber cotyledons. Isolation and analysis were performed as for Fig. 1.

Fig. 3 Agarose gel electrophoresis of *EcoRI* digests of *a*, cp-DNA isolated from normal *T. aestivum* var. Gaines (cytoplasm *T. aestivum*); *b*, cp-DNA isolated from cytoplasmic male-sterile *T. aestivum* var. Gaines (cytoplasm *T. timophevi*); *c*, mt-DNA from normal *T. aestivum* var. Gaines; *d*, mt-DNA from cytoplasmic male-sterile *T. aestivum* var. Gaines. Isolation and analysis were performed as described in Fig. 1.



gels *a*–*c*); this identity is direct evidence for the isocyttoplasmic origin of these varieties, deduced from interspecific reciprocal crosses¹². The corresponding cp-DNAs also led to identical patterns (results not shown).

The sensitivity of this method is illustrated on Fig. 2, gels (*d*) and (*e*), where mt-DNAs extracted from normal and tumorous cell suspensions of virginia creeper differ only by one band among 50; whereas this difference is highly reproducible, it is not known however whether this DNA modification is related to the tumorous transformation or simply results from an *in-vitro* evolution of the strains. Gels (*f*) and (*g*) in Fig. 2 show that mt-DNAs extracted from two physiologically different organs, cotyledons and hypocotyls, of a given plant (cucumber) do not present any detectable difference.

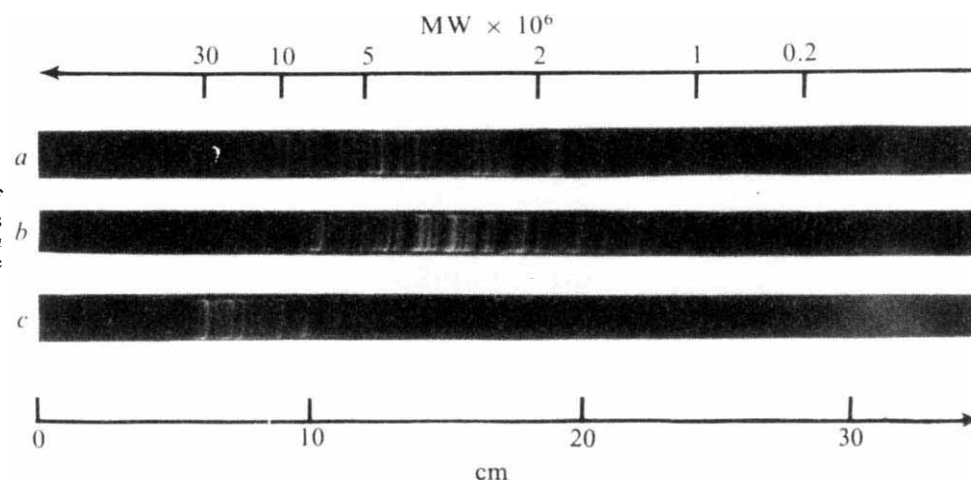
The cytoplasmic male sterility which affects many higher plant species has been claimed to result from mutation(s) in chloroplastic and/or mitochondrial DNAs (ref. 13). The mt-DNAs have been isolated from normal *T. aestivum* var. Gaines (cytoplasm *T. aestivum*) and from cytoplasmic male sterile *T. aestivum* var. Gaines (cytoplasm *T. timophevi*). Figure 3 *c*, *d* shows that the male sterile mt-DNA is altered for several bands; such a difference has been recently reported for cytoplasmic male sterile maize¹⁴. Since we have checked here that cp-DNAs of normal and male sterile lines are identical (Fig. 3, *a*, *b*) the male sterility mechanism seems to only involve the mitochondria.

All these mt-DNAs led to about 50 bands when split by *EcoRI*; this number is quite larger than that obtained with cp-DNAs, as illustrated on Fig. 1 (see ref. 6). The fluorescence intensity of several bands is greater than expected from normal stoichiometry and reflects the presence of multiple fragments. Even when these band multiplicities are not taken into account, the sum of the band molecular sizes estimated for virginia creeper, cucumber and wheat mt-DNAs were respectively

165×10^6 , 120×10^6 and 140×10^6 ; these values are much larger than the native molecular weight (60 to 70×10^6) directly determined from the contour length by electron microscopy (pea³; virginia creeper, our unpublished results). The sum is only 90×10^6 for potato calluses mt-DNA, probably because of the presence of a multiple band of high molecular weight (first band on Fig. 1 gel *c*). This value, 90×10^6 , is nevertheless larger than the native molecular weight estimated from the contour length (60×10^6 , data not shown). All these banding patterns were rigorously reproducible in respect to both the relative position and the relative intensity of the bands; several DNA isolations, different batches of enzyme, different concentrations of enzyme, extended digestion time (12 h), phenol-treated digests and digests heated to 60°C gave identical distributions. These electrophoregrams therefore represent non-reassociated limit digests.

The mt-DNA of *T. aestivum* var. Capitole has been exhaustively digested (7 h) by two other restriction endonucleases, *BamI* and *SalI*. The electrophoretic patterns are shown in Fig 4 and confirm that the sum of the fragment sizes remains quite larger than the native molecular weight, even when band multiplicities are not taken into account. The possibility that such discrepancies could result from a methylation cannot be ruled out, since methylation of a base in either strand of the endonuclease recognition site is sufficient to prevent the endonuclease from attacking the sequence¹⁵. Whereas it is agreed that cp-DNA does not contain 5-methylcytosine¹⁶, no information is available about the presence of methylated bases into the mt-DNA of higher plants. This methylation should not occur randomly since the electrophoretic pattern remains unaltered (in number and size of fragments and in relative intensity of band fluorescence) for a given plant species, whatever the organ, the age of the plant or the growth conditions (data not shown).

Fig. 4 Agarose gel electrophoresis of *EcoRI* (*a*), *BamI* (*b*), and *SalI* (*c*) digests of mt-DNA isolated from *T. aestivum* var. Capitole. Experimental procedure as for Fig. 1.



If methylation is to be ruled out, the main conclusion arising from above molecular weight discrepancies should be that the population of mt-DNA molecules is heterogeneous and that an higher plant contains different mt-DNA molecules. The plant mitochondrial genome would be constituted by several circular mt-DNA molecules with identical buoyant density but with some differences in sequence arrangement. The mitochondrial genome complement would be represented either by one mitochondria containing different circular mt-DNA molecules or by several classes of mitochondria¹⁷, each containing one type of DNA molecule.

Whereas inter- and intraspecies variations have been detected for mammalian mt-DNAs, the mt-DNA of each studied mammal seems to be constituted by a single molecular clone, as evidenced by the good correspondence between the contour length and the molecular weight estimated from *Hae*III restriction patterns¹⁸. Higher plant mitochondria thus differ from mammalian mitochondria in this respect. Heterogeneity of this type has been reported for the kinetoplast DNA isolated from *Criothidia luciliae* and split by *Hap*II restriction nuclease¹⁹.

The determination of mt-DNA restriction patterns, together with study of the chloroplastic DNA (ref. 6), should be very useful in analysis of phylogenies and in determining the nature of parasexual hybrids.

We thank Drs Y. Cauderon and P. Auriau (INRA, Versailles) for seeds of *T. aestivum* var. Gaines, and Dr Hoffnung (Institut Pasteur, Paris) for *Sal*I.

FRANCIS QUÉTIER
FERNAND VEDEL

Laboratoire de Biologie Moléculaire Végétale,
Associé au CNRS (L.A. 40), Bât. 430,
Université Paris-Sud,
91405 Orsay, France

Received 5 January; accepted 3 June 1977.

- Wolstenholme, D. R. & Gross, N. J. *Proc. Natn. Acad. Sci. U.S.A.* **61**, 245-252 (1968).
- Wells, R. & Birnstiel, M. *Biochem. J.* **112**, 777-786 (1969).
- Kolodner, R. & Tewari, K. K. *Proc. Natn. Acad. Sci. U.S.A.* **69**, 1830-1834 (1972).
- Vedel, F. & Quétier, F. *Biochim. biophys. Acta* **340**, 374-387 (1974).
- Wells, R. & Ingle, J. *Pl. Physiol.* **46**, 178-179 (1970).
- Vedel, F., Quétier, F. & Bayen, M. *Nature* **263**, 440-442 (1976).
- Antis, P. T. P. & Northcote, D. H. *J. exp. Bot.* **24**, 425-441 (1973).
- Grienenberger, J. M., Quétier, F. & Vedel, F. *Pl. Sci. Lett.* **6**, 379-388 (1976).
- Davis, R. W., Simon, M. N. & Davidson, N. *Meth. Enzym.* **21**, 413-428 (1971).
- Kolodner, R. & Tewari, K. K. *Biochim. biophys. Acta* **402**, 372-390 (1975).
- Thomas, M. & Davis, R. W. *J. molec. Biol.* **91**, 315-328 (1975).
- Kihara, H. *Seiken Zoho* **22**, 107-111 (1971).
- Flavell, R. B. *Pl. Sci. Lett.* **3**, 259-263 (1974).
- Levings, C. S. & Pring, D. R. *Science* **193**, 158-160 (1976).
- Arber, W. & Linn, S. A. *Rev. Biochem.* **38**, 467-500 (1969).
- Baxter, R. & Kirk, J. T. O. *Nature* **222**, 272-273 (1969).
- Fletcher, J. S. *Nature* **238**, 466-467 (1972).
- Potter, S. S., Newbold, J. E., Hutchison III, C. A. & Edgell, M. H. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4496-4500 (1975).
- Kleisen, C. M. & Borst, P. *Biochim. biophys. Acta* **407**, 473-478 (1975).

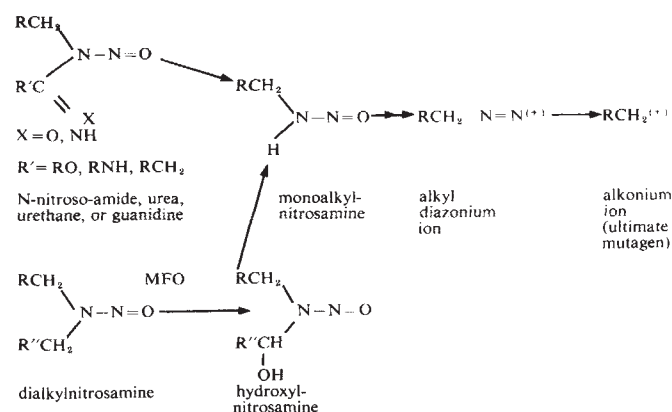
Inhibition by L-ascorbate of bacterial mutagenesis induced by two *N*-nitroso compounds

MANY chemicals are activated to carcinogens and mutagens by oxidative metabolism to alkylating agents¹. Ascorbic acid has been reported to protect against tumour induction in mice by 3-hydroxyanthranilic acid, which induced bladder tumours² and 7,12-dimethylbenzanthracene plus croton oil which induced skin tumours³. Ascorbic acid can also prevent the formation of carcinogenic *N*-nitroso compounds from nitrite and amines, *in vivo*⁴ and *in vitro*⁵. I report here that mutagenesis by two preformed *N*-nitroso compounds is effectively inhibited by ascorbic acid.

Many *N*-nitroso compounds are potent carcinogens and mutagens⁶⁻⁸. The mechanism of action of these compounds is believed to involve decomposition to short-lived highly reactive electrophiles which react with nucleophilic sites on DNA bases leading to altered bases⁶⁻⁸. Some of these alterations may not be fully repairable in certain tissues, and cause base

pair substitution mutations, and perhaps lead to tumour formation^{9,10}. There is evidence that the reactive electrophile and ultimate mutagen is an alkonium ion, which is formed by rapid intramolecular rearrangements subsequent to spontaneous scission of the derivatised α carbon of *N*-nitroso-amides, ureas, urethanes or guanidines⁶⁻⁸. *N*-nitrosamines also decompose to yield alkonium ions, but as their *N*- α -carbon bonds are not labile, nitrosamines must be metabolically activated by an α -hydroxylase activity present in the mixed function oxidase (MFO) system⁶⁻⁸.

These reaction pathways are depicted below:



As both types of *N*-nitroso compounds are believed to generate altered bases by an electrophilic attack on nucleophilic sites in DNA, it seems possible that an exogenous nucleophile could compete with DNA bases for the ultimate mutagen and thus serve as a possible preventative agent against mutagenesis and carcinogenesis. As tissue and biological fluids contain nucleophilic compounds which could also react with alkonium ions, this nucleophile would have to be more reactive than these endogenous nucleophiles if it were to be effective. Alternatively, it could be of comparable nucleophilicity but be present in higher concentrations than endogenous nucleophiles in the proximity of DNA. It should also be non-toxic at the levels used. One such compound might be L-ascorbic acid. Although usually known for its anti-oxidant properties, L-ascorbate which is the predominant form at physiological pH, possesses substantial nucleophilic character¹¹. In fact, it has been suggested by Edgar that ascorbate might protect against electrophilic attack on cellular DNA by intercepting alkylating agents¹¹. In addition, it can be ingested in moderate quantities without apparent toxicity^{12,13}. In this study the effects of ascorbate ion on microbial mutagenesis by *N*-methyl-*N*-nitrosoguanidine (MNNG) and dimethylnitrosamine (DMN), were examined.

Mutagenesis was assayed by a bacterial auxotroph reversion test using the histidine requiring *Salmonella typhimurium* tester strain TA 1530 described by Ames *et al.*¹⁴. The microorganisms were incubated with MNNG or DMN in 0.1 M phosphate buffer, pH 7.4, in the presence or absence of ascorbic acid. When DMN was used, a rat liver extract, containing the MFO system was incorporated into the incubation mix¹⁵. Aliquots were taken at several incubation times and reversion of microorganisms to histidine independence was assayed as described in Fig. 1.

In Fig. 1 the inhibitory effects on MNNG mediated mutagenesis by various concentrations of ascorbic acid are shown. Higher concentrations of ascorbate caused greater inhibition of mutagenesis. Mutagenesis by DMN, (Fig. 1c, d), is also inhibited by ascorbate. Higher concentrations of ascorbate are necessary to inhibit mutagenesis by DMN than by MNNG. The concentration of ascorbate necessary to produce a particular percentage inhibition of DMN-induced mutagenesis is a function of the concentration of liver extract in the incubation mixture. For example, when DMN was activated by

1.5 mg ml⁻¹ liver extract, 90% inhibition was afforded by 1.5 mg ml⁻¹ ascorbate. When twice the concentration of liver extract was used and other conditions held constant about twice the concentration of ascorbate was required for the same percentage inhibition. As both DMN and MNNG are believed to yield the same ultimate mutagen⁶, it seems that the components of the liver extract rendered ascorbate a less effective inhibitor, since the MNNG mutagenesis system contained no added activation system. Since proteins contain a number of side chains with nucleophilic moieties it is possible that some of these may compete with ascorbate by reacting with the ultimate mutagen, thus decreasing the life-time of the mutagen. This effect seems to be important since added bovine serum albumin, 2 mg ml⁻¹ in the absence of ascorbate, inhibited mutagenesis by MNNG by over 90%.

Alternatively the liver extracts might bind or deactivate ascorbate and thus lower its effective concentration. Some indirect evidence of this latter effect may be seen in the fact that in the absence of liver extracts ascorbic acid is toxic to the bacteria at concentrations above 0.3 mg ml⁻¹, but in the presence of liver extracts no toxicity is observed at concentrations as high as 3.0 mg ml⁻¹. Ascorbate toxicity has been reported with other strains of bacteria¹⁶. No significant toxicity was observed in the conditions used in this study. In view of these complications and the possibility of ascorbate interference in the activation of DMN to a mutagen, further studies were carried out with simpler MNNG system.

If there is in fact competition between ascorbate and micro-organisms for the ultimate mutagen, then a further demonstration of this competition would be a decreased efficiency of inhibition by ascorbate at higher concentrations of micro-organisms. As shown in Table 1, the percentage inhibition by

Table 1 Effect of concentration of bacteria on inhibition of MNNG mediated mutagenesis by ascorbate

Bacteria ($\times 10^8$ c.f.u. per m)	Revertants ml ⁻¹		R_a/R_0
	No ascorbate (R_0)	Ascorbate (R_a)	
2.6	3,380	300	0.088
4.6	4,840	1,260	0.26
6.5	11,010	3,590	0.33
13.0	14,020	6,250	0.45

Mutagenesis was assayed as described in Fig. 1. The incubation time was 15 min, the concentration of MNNG was 8.0 μ M and that of ascorbic acid 0.06 mg ml⁻¹ c.f.u., Colony forming units.

ascorbate drops as the concentration of bacteria in the incubation mix increases. This effect and the increasing inhibition at higher ascorbate concentrations described above suggest that ascorbate exerts its inhibitory effect by trapping the ultimate mutagen before it interacts with cellular DNA.

Other biological nucleophiles also inhibited mutagenesis by MNNG (see Table 2). But none of those tested was as effective as ascorbate. The compounds tested were chosen because they contain most of the functional groups that are found in significant concentrations in biological fluids¹⁷. The actual concentrations of these compounds relative to ascorbate will vary in different tissues and with ascorbate intake. In urine for instance, ascorbate concentrations may be considerably higher than those used in this study¹⁸, whereas the concentrations of the compounds in Table 2 are usually significantly lower¹⁷.

The concentration of ascorbate necessary to effectively inhibit mutagenesis by MNNG can readily be exceeded in the urine¹⁸, and may be reached in certain other tissues¹⁸. As this study indicates, a number of compounds normally found in urine are capable of inhibiting mutagenesis by MNNG; however, ascorbate is the most effective. It cannot be excluded, however, that ascorbate might be able to deactivate the parent *N*-nitroso compounds by some other mechanism such as reduction, which is not available to most of the compounds listed in Table 2. Several *N*-nitroso compounds are known bladder carcinogens in animals⁸. If the mechanisms of carcinogenesis and mutagenesis are similar¹⁹, and if *N*-nitroso compounds are bladder carcinogens in humans, then some protective effect against these compounds might be afforded by saturating doses of vitamin C. In previous studies where ascorbate inhibited carcinogenesis its protective role was attributed to its role in preventing oxidative activation of carcinogens^{2,3}, presumably to electrophiles. It is possible that ascorbate may also exert a protective effect by intercepting electrophilic

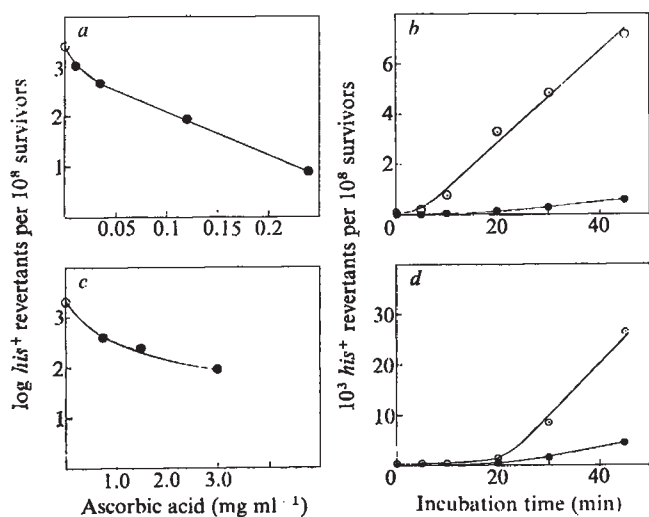


Fig. 1 Effect of ascorbate on mutagenesis of *Salmonella typhimurium* TA 1530 by MNNG and DMN. *a, c*, Effect of concentration of ascorbate on mutagenesis by MNNG and DMN respectively; *b, d*, effect of incubation time on mutagenesis by MNNG and DMN respectively. Reaction mixtures (0.5 ml) contained: bacteria, 2×10^8 c.f.u. centrifuged from a stationary phase culture; MNNG, 8.0 μ M (*a*), 13 μ M (*b*) or DMN 200 mM + liver extract from Aroclor pretreated rats¹⁶, containing 1.5 mg ml⁻¹ protein (*c*) or 3.0 mg ml⁻¹ (*d*); 0.1 M phosphate buffer (pH 7.4); ascorbic acid, 0.12 mg ml⁻¹ (*b*), 3.0 mg ml⁻¹ (*d*) or varying concentrations (*a*) and (*c*). $\circ$, no ascorbate; $\bullet$, ascorbate. Test tubes containing the incubation mix were shaken aerobically at 37°C, for 15 min (*a*), 40 min (*c*) or varying times, (*b*) and (*d*). Bacteria were assayed for histidine independent (*his*⁺) revertants and cell viability. Cell viability was not significantly decreased during the incubation. The spontaneous revertant frequency was < 10 per 10^8 c.f.u. and was not subtracted from the total revertants. Ascorbate may have been slightly mutagenic, as in the absence of added mutagen it caused a doubling in the revertant frequency. Revertant frequencies obtained from duplicate assays were within 15% of each other. *S. typhimurium* TA 1530 was kindly provided by B. Ames.

Table 2 Effects of various biological compounds on mutagenesis by MNNG

Addition (mM)	10 ³ Revertants
	10 ⁸ c.f.u.
Ascorbic acid, 6.8	3.0
Creatine-H ₂ O, 8.1	0.04
Cysteine-HCl, 7.6*	0.69
Methionine, 8.1	3.3
Serine, 11.4	2.5
Tyrosine, 6.6	0.43
Tryptophan, 5.9	0.62
	0.54

Mutagenesis assay was as described in Fig. 1. The incubation time was 8 min and the concentration of MNNG was 8.0 μ M. All additions were present at a final concentration of 0.12 mg ml⁻¹. Similar percentage inhibition was seen at longer incubation times.

*Cysteine became inhibitory at longer incubation times. At 2 h incubation when mutagenesis had nearly ceased it had inhibited mutagenesis by 50%. It was the only compound in this table to show this effect.

metabolites, or their precursors, independent of their mode of production.

I thank Miriam Miranda for technical assistance. This work was supported by the USPHS.

Note added in proof: Recent experiments have shown that addition of ascorbate to solutions of MNNG causes a rapid decrease in the absorbance of MNNG at 400 nm, whereas addition of the same concentrations of the other compounds in Table 2 had little effect. Some of the MNNG may thus be directly deactivated by ascorbate.

JOSEPH B. GUTTENPLAN

New York University Dental Center,
New York, New York 10010

Received 7 April; accepted 31 May 1977.

- 1 Miller, J. A. *Cancer Res.* 30, 559-576 (1970).
- 2 Schlegel, J. U., Pipkin, G. E., Nishimura, R. & Schultz, G. N. *J. Urol.* 103, 155-159 (1970).
- 3 Shamberger, R. J. *J. natn. Cancer Inst.* 48, 1491-1495 (1972).
- 4 Kamm, J. J., Dashman, T., Conney, A. H. & Burns, J. J. *Ann. N.Y. Acad. Sci.* 258, 169-174 (1975).
- 5 Mirvish, S. S. *Ann. N.Y. Acad. Sci.* 258, 175-180 (1975).
- 6 Druckery, H., Preussman, R., Ivankovic, S. & Schmaehl, D. Z. *Krebsforschung* 64, 103-210 (1967).
- 7 Magee, P. N. & Barnes, J. M. *Adv. Cancer Res.* 10, 163-246 (1967).
- 8 Magee, P. N., Montesano, R. & Preussman, R. in *Am. Chem. Soc. Monograph, Chemical Carcinogens* (ed. Scarle, C.) (1976).
- 9 Lovelass, A. *Nature* 223, 206-207 (1969).
- 10 Nicoll, J. W., Swann, P. F. & Pegg, A. E. *Nature* 254, 261-262 (1975).
- 11 Edgar, J. A. *Nature* 248, 136-137 (1974).
- 12 Lamden, M. P. & Chrystowski, G. A. *Proc. Soc. exp. Biol. Mol.* 85, 190-192 (1954).
- 13 Anderson, T. W. *Ann. N.Y. Acad. Sci.* 258, 498-503 (1975).
- 14 Ames, B. N., Lee, F. D. & Durston, W. E. *Proc. natn. Acad. Sci. U.S.A.* 70, 872-876 (1973).
- 15 Garro, A. J., Guttenplan, J. B. & Milvy, P. *Mutat. Res.* 38, 81-88 (1976).
- 16 Pauling, L. *Proc. natn. Acad. Sci. U.S.A.* 71, 4442-4446 (1976).
- 17 Altman, P. L. & Dittmer, D. S. *Blood and Other Body Fluids* 363-370 (Federation of American Societies for Experimental Biology, Washington DC, 1961).
- 18 Burch, H. B. *Ann. N.Y. Acad. Sci.* 92, 268-276 (1961).
- 19 McCann, J., Choi, E., Yamasaki, E. & Ames, B. N. *Proc. natn. Acad. Sci. U.S.A.* 72, 5135-5139 (1975).

Intracellular cationic counterion composition of an acid mucopolysaccharide

EVEN though much is known about biopolymer synthesis and secretion, little or nothing is known about the exact identity of counterions bound to charged biopolymers inside cells. This may be very important since bound ions can influence polymer size and shape and so may hold answers to questions about polymer packaging in cells. In this paper we demonstrate how X-ray microprobe analysis of frozen mucus cells, from a marine snail, has enabled us to show that a sulphated mucopolysaccharide only binds potassium inside the cell but releases this to bind magnesium and calcium after secretion. This is of particular interest because, of all the common physiological cations, only potassium precipitates this mucopolysaccharide *in vitro*. The implication is that potassium could cause the polymer to adopt a more compact form within the cell.

Fig. 1 Scanning electron micrograph of a freeze-fractured face in the hypobranchial gland epithelium of the gastropod *Buccinum undatum*. Arrow A points to an acid mucopolysaccharide cell filled with vesicles while arrow B indicates an area of ciliated and interstitial cells. Both areas A and B were probed for elemental analysis by X-ray emission. The results are shown in Fig. 2 and Table 1. Probe spot diameters of about 2 µm were used.

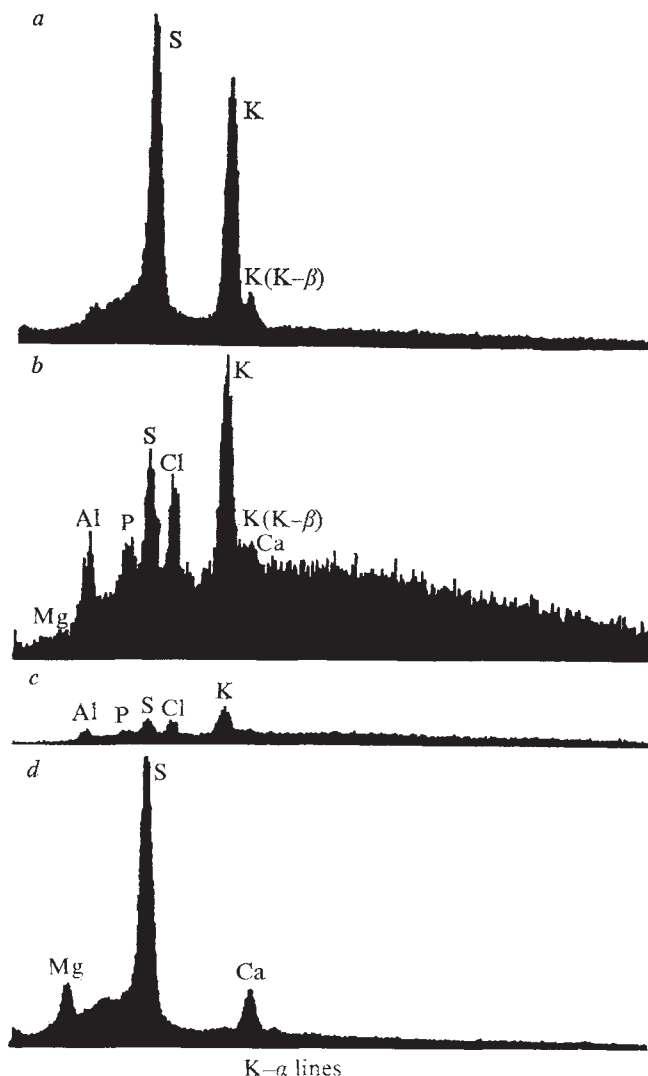
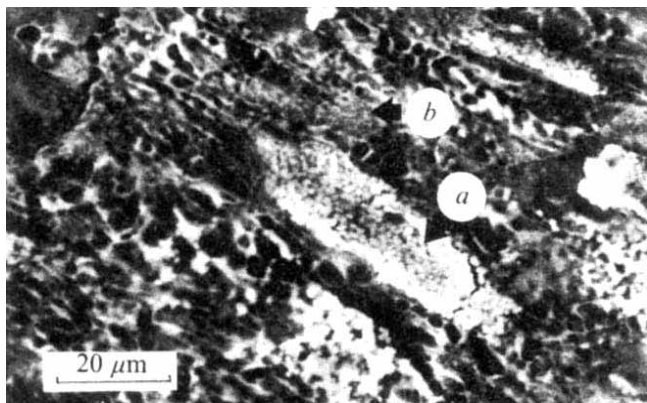


Fig. 2 Energy dispersive X-ray analyses of cells and mucopolysaccharide in the hypobranchial gland of *Buccinum undatum*. *a*, Acid mucopolysaccharide cell A shown in Fig. 1. *b*, Area of ciliated and interstitial cells shown at B in Fig. 1. *c*, Plot 2b at the same scale expansion as plot 2a. *d*, Whole mucin collected in seawater at the gland surface and dialysed extensively against distilled water. Aluminium is instrumental in origin. All emission bands are K- α in character with the exception of the weak potassium K- β which is only seen when the potassium level in the specimen is very great.

Cells synthesising and exporting acid mucopolysaccharides, such as for example the cells of vertebrate cartilage or the epithelial gland cells of many marine invertebrates, must experience a problem of cationic balance. From the moment that the polymer first receives its charged groups, whether these be carboxyl or sulphate, the intense electrostatic field generated by the polyanion will attract an atmosphere in which cations will predominate; moreover, as with polyelectrolytes generally, a substantial proportion of these cations will be bound specifically as ion pairs. There is evidence indicating that there may be orders of preference of binding different cations by differing anionic groups^{1,2} and suggesting that different polymers may show different cation specificities². The type of counterion bound may in some cases radically affect the polyanion conformation³.

Whether polyanion synthesis is accompanied by a continuous export from the cell or by storage in vacuoles for bulk secretion, the cytoplasm of the cells would experience an unusual demand on the free cation pool, which has to be restored by inward transport of cations. Moreover, should the polyanion show cation specificity, unusual imbalance in the cation composition of the cell may develop with obvious

implications. These are possibilities which can only be speculated on in the absence of information about the counterion distribution surrounding polyanions.

Although determination of the ion-binding properties of an acid mucopolysaccharide outside the cell is relatively easy, it has hitherto been impossible to determine the intracellular counterion distribution accompanying the polyanion. We have now carried out such an analytical electron microscopic study using a secretory epithelium from the marine gastropod *Buccinum undatum*. We chose this tissue rather than vertebrate connective tissue cells for an initial investigation because the polysaccharide produced is fairly simple, containing only ester sulphate and no carboxyl groups^{4,5}, the tissue has been well described ultra-structurally⁶, the secretion vesicles inside the cell are readily seen in the electron microscope⁹ and finally, because the presence of ester sulphate and little or no amino acid sulphur in the mucopolysaccharide allows for straight comparison between sulphur and cation X-ray signals in the analytical microscope. We used the hypobranchial gland, which secretes a mucin into the mantle cavity of the mollusc and is a folded epithelium with a large population of secretory and ciliated cells. Mucin forms by the mixing of two secretion products at the gland surface. One product, the polyanion, is essentially cellulose with one ester sulphate residue per glucose unit^{4,5}, and the other, formed in a different cell type⁶, is a neutral glycoprotein.

Since any form of fixation, dehydration and embedding would have radical effects on cation content in the tissue we adopted the strategy of quench-freezing freshly excised tissue in 'slushy' liquid nitrogen, placing the frozen specimen in the scanning electron microscope and cleaving the tissue on the freezing stage *in vacuo* with a scalpel blade. The cells are exposed in fractured section and vacuum sublimation sufficiently etches the cleft surface to make tissue topography readily recognisable when compared with conventional micrographs⁶. Figure 1 shows such a cleft surface, acid mucopolysaccharide cells are clearly distinguishable from other cells. Electron beam probing and energy dispersive X-ray analysis applied to the secretion vacuoles of these cells gave the results typified in Fig. 2a. Interestingly high sulphur and potassium peaks dominated the spectrum and no other cation peaks were seen in these plots. Probing non-mucopolysaccharide cells in the tissue gave results typified by Fig. 2b and c. These are shown at the same and greater scale expansion as the acid mucopolysaccharide cells, to emphasise the high levels of potassium and sulphur present in the secretory vacuoles relative to the ionic content of the general cytoplasm of other cells.

Since we had shown that the polyanion probably exists in the cell solely as the potassium salt, we tried to determine whether the counterion is altered when the polyanion is released into the seawater medium. It is at this time also

that polyanion and glycoprotein interact to form a rheotropic mucin. Figure 2d shows the X-ray analysis of a mucin sample collected under seawater as it left the gland. The mucin had been transferred immediately to distilled water, dialysed extensively and freeze-dried. Potassium has gone from the trace completely and has been replaced by magnesium and calcium. Sodium too, is absent. Dialysis of the mucin against seawater before distilled water does not alter this pattern.

A difficulty about this technique is its semi-quantitative nature. X-ray counting efficiency falls off rapidly for atomic numbers below 15 and so on an atom-to-atom basis sulphur and sodium peaks should be in approximately 10:1 ratio, whereas equivalent heights of sulphur and potassium peaks are more nearly indicative of equivalent atomic ratios. Nevertheless, the height of the magnesium peak indicates that sodium would be detected if present.

In Table 1 we show the integrated values of the elemental peaks seen in Fig. 2a-c as well as values for scans of the purified acid mucopolysaccharide as its potassium and sodium salts and in the ionic form generated by dialysis of the potassium salt against seawater. Attempts to replace potassium by sodium using high salt concentrations or sodium-form ion-exchange resins fail to remove all potassium. The reverse is not true. Like whole mucin, pure polyanion exposed to seawater loses all detectable monovalent cation, gaining mainly magnesium and somewhat less calcium.

Thus it seems that the polyanion is synthesised and stored as the potassium salt but changes in the sea to the magnesium-calcium form, in spite of the availability of sodium, magnesium and calcium inside the cell. Intracellular ionic compositions are difficult to obtain, depending on knowledge of extracellular space volumes. As an example, however, we quote analyses⁷ of squid muscle cells with values of 30.8, 189, 1.88 and 19.0 mg ions per kg water for Na, K, Ca, Mg respectively, with seawater containing 492, 10.5, 10.8 and 56.1 mg ions per kg respectively of the same ions. The high concentration of sodium in seawater seems not to affect the ability of the polyanion to bind divalent ions which also compete very successfully with potassium. Since magnesium and calcium, albeit at low concentrations, are probably present in the cell why do they not also compete there with potassium for binding sites? Of interest here is the fact that concentrations of potassium ions in excess of 0.2 M *in vitro* precipitate the polysaccharide sulphate and this we use as a purification step¹. This property is shared by some other polysaccharide sulphates, for example, K-carageenan⁸. Excess concentrations of sodium, magnesium or calcium salts do not have this effect.

Packaging is important for the cell since most acid mucopolysaccharides hydrate readily and attempt to occupy large

Table 1 Integrated elemental X-ray counts for tissue and isolated acid mucopolysaccharide preparations from the hypobranchial gland of *Buccinum undatum*

	Na	Mg	P	S	Cl	K	Ca	Scale expansion
Acid mucopolysaccharide cell (Fig. 1)	0	0	0	26,564	0	20,559	0	4
Non-secretory cell (Fig. 1)	not measurable	120	300	1,259	995	2,122	210	7
Whole mucin after secretion into seawater	0	5,630	0	49,538	0	0	8,000	3
Acid mucopolysaccharide dialysed against seawater	0	4,669	0	63,724	0	0	7,108	3
Acid mucopolysaccharide, potassium form	0	0	0	49,524	0	38,784	0	3
Acid mucopolysaccharide, sodium form	13,860	0	0	130,728	0	6,602	0	2

X-ray energy dispersive spectra were obtained using a Jeol JSM 50A scanning electron microscope in conjunction with a Kevex analyser. Specimens were mounted on carbon stubs and maintained in the microscope at a stage temperature of -104°C . Exciting voltages, count times and take off angles were held constant at values of 20 KV, 100 s and 30° respectively for all specimens. Vertical scale expansions were varied to accommodate X-ray yields (which are sensitive to specimen thickness and density) at major elemental peak heights. In the case of non-secretory cells X-ray continuum made sodium counting of the low levels present non-productive.

We thank Professor Charles Phelps and Drs Ian Nieduszynski and John Sheenhan for helpful discussions.

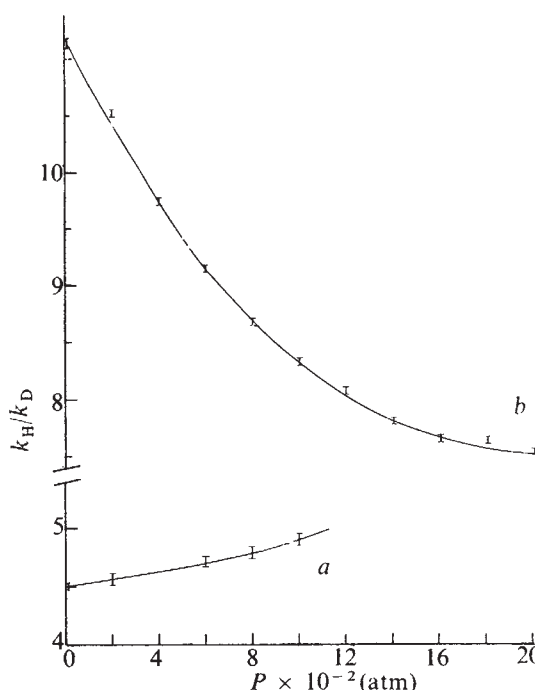
*Department of Biological Sciences,
University of Lancaster,
Bailrigg, Lancaster, UK*

- ¹ Bungenberg de Jong, H. G. in *Colloid Science* (ed. Kruyt, H. R.) 2 (Elsevier, Amsterdam, 1949).
- ² Scott, J. E. in *The Chemical Physiology of Mucopolysaccharides* (ed. Quintarelli, G.) 171-187 (Little, Brown and Co., Boston, 1968).
- ³ Atkins, E. D. T., Sasse, H. J., Huclozyski, I. A., Phelps, C. F. & Sheehan, J. L. *Polym. J.* 5, 263-271 (1974).
- ⁴ Hunt, S. & Jevons, F. R. *Biochem. J.* 98, 522-529 (1966).
- ⁵ Hunt, S. *Polysaccharide-Protein Complexes in Invertebrates* (Academic, London & New York, 1970).
- ⁶ Hunt, S. *J. mar. biol. Ass. U.K.* 53, 59-71 (1973).
- ⁷ Robertson, J. D. *J. exp. Biol.* 42, 153-175 (1965).
- ⁸ Baylev, S. T. *Biochim biophys. Acta* 17, 194-196 (1955).

WE report here the first measurement of the effect of pressure on primary kinetic isotope effects of reactions in the liquid phase. We have determined rates of two reactions in solution by a sampling technique at pressures up to 2 kbar using both normal and deuterium-labelled reagents as indicated. Reaction (1) is between benzoic acid and diphenyldiazomethane (I) which has been shown to proceed by a rate-determining proton transfer^{1,2}. Reaction (2) is between leuco crystal violet (II) and chloranil (III) in which the slow step is a hydride transfer³.



We find that the behaviour of the two isotope effects under pressure is quite different. That of reaction (1) remains almost invariant while that of (2) smoothly decreases until at 2 kbar it levels off at a value near the maximum 'normal' ratio of about



7.5 (Fig. 1). It follows that the volumes of activation, $\Delta V^\ddagger$, calculated (using the regression equation $\ln k = A - \Delta V^\ddagger/RT + CP^\ddagger$) for reactions (1) (H) and (1) (D) are the same ($-12.8 \text{ cm}^3 \text{ mol}^{-1}$) while those of reactions (2) (H) and (2) (D) are very divergent, being -25.5 and $-35.8 \text{ cm}^3 \text{ mol}^{-1}$ respectively.

It is assumed with good justification that isotopic substitution does not alter the reaction pathway or position of the transition state along the reaction coordinate⁶; therefore it seems likely that the value of $\Delta V^\ddagger$ for reaction (2) (H) is anomalous because of a tunnelling component. In this reaction external pressure serves to lower the potential barrier to the activation process. Consequently a higher proportion of reagents will be capable of surmounting the barrier normally and the relative importance of tunnelling will diminish with increasing pressure analogous to the effect of an increase in temperature⁷. This analysis suggests that the tunnelling contribution of a reaction with a positive volume of activation (that is, which has a rate decreasing with pressure) and consequently the isotope effect, should increase with pressure. In general, where tunnelling is important, $\Delta V^\ddagger(\text{H}) < \Delta V^\ddagger(\text{D})$.

Although more data are needed to give confidence to this interpretation the result suggests that pressure dependence of the isotope effect may serve as a further criterion of the occurrence of tunnelling in hydrogen-transfer reactions.

Chemistry Department,
University of Reading,
Whiteknights, Reading, UK

Received 2 May; accepted 18 May 1977.

- ¹ Chapman, N. B., Dack, M. R. J., Newman, D. J., Shorter, J. & Wilkinson, R. *J. Chem. Soc. (Perkin II)* **962**, 971 (1974).
- ² Isaacs, N. S. & Rannala, E. *J. Chem. Soc. (Perkin II)* **962**, 899 (1974).
- ³ Bromberg, A., Muszkat, K. & Fischer, E. *Chem. Commun.* 1352 (1968).
- ⁴ Melander, L. *Isotope Effects on Reaction Rates* (Ronald, New York, 1960).
- ⁵ Lewis, E. S., Perry, J. M. & Grinstein, R. H. *J. Am. Chem. Soc.* **92**, 899 (1970).
- ⁶ Westheimer, F. H. *Chem. Rev.* **61**, 265 (1961).
- ⁷ Bell, R. P. *The Proton in Chemistry*, 2nd edn (Chapman and Hall, London, 1973).

matters arising

Generalisation of self-thinning of plant populations

WESTOBY'S attempt¹ to generalise self-thinning of plant populations by relating leaf area per plant (L) to surviving plant density (p) is spurious. The result, therefore, is not of 'more general application than the original statement of the rule'².

The measured value of leaf area index (LAI) in many plant stands may vary from <1 to 20, but rarely exceeds 10 (refs 3, 4, 5). Westoby's Fig. 3 shows not merely an error in graphical labelling (the ordinate values are 10 times too large), but reveals a more fundamental misconception about the dynamics of thinning. The relationships between leaf area (L) and dry weight (W) shown in Fig. 2 are not applicable to the experimental circumstances depicted in Fig. 1 on at least two grounds. Firstly, Blackman & Wilson⁶ derived their leaf area ratios ($LAR = L/W$) from spaced plants (~ 20 plants m^{-2}). LAR varies significantly with density^{3,7,8} and with light intensity⁶ (as Fig. 2 shows) and may vary with age⁹. Secondly, the range of W in Fig. 2 seldom exceeds 1.0 g, whereas its range in Fig. 1 is generally an order of magnitude greater: the extrapolation of equations derived from Fig. 2 over this range is unjustified. The neglect of density effects on LAR , coupled with graphical extrapolation cause the error in Fig. 3.

The behaviour of LAI in plant stands (both of herbs and trees) is simply stated: it rises rapidly, as a function of density and light intensity *inter alia*, to a plateau value, which is commonly <10 . Thinning may occur while LAI is increasing and/or for a prolonged period while LAI remains constant, or even declines. The most general statement of the dynamics is $L = K^{-1.0}$, where K varies significantly with light intensity. This is shown very clearly for *Helianthus annuus* in the original source from which Fig 1 was derived: 'the convergent leaf area index at 100%, 60% and 23% daylight was respectively 8, 6.5 and 1.5' (ref. 3), an empirical result which belies Fig. 3, where stands with least light have highest LAI for a given chronological age!

It should also be noted that the author's use of K in both equations

relating W and L to p is misleading, as it implies similar constants: they are quite different.

JAMES WHITE

Department of Botany,
University College,
Belfield, Dublin 4,
Ireland

¹ Westoby, M. *Nature* 265, 330-331 (1977).

² Moore, P. D. *Nature* 265, 295 (1977).

³ Hiroi, T. & Monsi, M. *J. Fac. Sci. Univ. Tokyo III* 9, 241-285 (1966).

⁴ Monteith, J. L. in *Physiological Aspects of Crop Yield* (eds Eastin, J. D. et al.) 89-111 (American Society of Agronomy, Madison, 1969).

⁵ Art, H. W. & Marks, P. L. *XVth I.U.F.R.O. Congress, Sect. 25 (Growth & Yield)* 3-32 (1971).

⁶ Blackman, G. E. & Wilson, G. L. *Ann. Bot. N.S.* 15, 63-94 (1951).

⁷ Lomms, R. S. et al. *Crop Sci.* 8, 352-356 (1968).

⁸ Hodanova, D. *Biol. Plant (Praha)* 9, 424-438 (1967).

⁹ Evans, G. C. *The Quantitative Analysis of Plant Growth* (Blackwell, Oxford, 1972).

WESTOBY REPLIES—I thank Dr White for his helpful remarks. Firstly, he is quite correct that the scaling of the y axis in my Fig. 3 was $\times 10$ too high; I apologise for confusing readers. Figure 3 was not intended as a simulacrum of a real experiment, but as a demonstration that the response of LAR to shading is the right kind to return the slope of a low-light thinning line to $-3/2$. That remains my basic point. Certainly LAR responds to planting density, among other factors. But does high planting density obliterate the response of LAR to shading?

I should like to suggest that perhaps the underlying determinant of relative mortality rate is how fast the light climate is deteriorating for the smallest plants in the population. Which descriptor of the population would correlate best with this process would depend on circumstances. During early growth biomass would be adequate unless LAR was varied by light treatments, in which case leaf area would be better. As the death rate of leaves rises LAI asymptotes, but the location of the canopy continues to rise, which has the effect of putting the smallest plants lower in the light profile. Here cumulative production of leaf area or biomass (as measured by Hiroi and Monsi) would be the most relevant descriptor. Where stem production is cumulative and comes to dominate the biomass (for example, in tree stands), stem weight or volume will be the best

available indicator of cumulative leaf area production.

MARK WESTOBY

School of Biological Sciences,
Macquarie University,
North Ryde, NSW 2113
Australia

Atlantic palaeotemperatures during the Cainozoic

IT is comparatively easy to make generalisations in palaeo-oceanography, and Cifelli¹ has made several postulates regarding the Cainozoic palaeotemperatures of the North Atlantic; but the test of speculation in palaeo-oceanography is detailed biostratigraphy. For example, Cifelli¹ postulated that during the Miocene, the North Atlantic Gyre "extended perhaps 10° further north than they do now". The limited evidence, by Berggren², DSDP Leg 12, Sites 116 and 117 hardly support this: Berggren's² zonation of the Miocene planktonic foraminifera is reminiscent of that established in the cooler higher latitudes of New Zealand^{3,4} than of the tropical belt zonations by Bolli⁵ and Blow⁶. Work recently completed⁷ on a cored Lower Miocene Burdigalian borehole sequence 110 km south-west of the Isles of Scilly has yielded an excellent planktonic foraminiferal fauna which lacks the following warm-water taxa recorded in Trinidad⁸: *Globigerina venezuelana* Hedberg, *Catapsydrax unicavus* Bolli, *C. dissimilis* (Cushman and Bermudez), *C. stainforthi* Bolli, *Globorotaloides suteri* Bolli, and *Globigerinatella insueta* Cushman and Stainforth. Conversely, the fauna contains cooler water modern species such as *Globigerina bulloides* d'Orbigny and *Turborotalita quinqueloba* (Natland) as well as cooler water extinct fossils such as *Globigerina woodi* Jenkins and *Globorotalia zealandica* (Hornibrook).

Thus from the limited, but accumulating evidence available, the planktonic foraminifera in Miocene of the North Atlantic indicate that near-surface palaeotemperatures of the ocean were not as warm as postulated by Cifelli¹.

There is well documented evidence⁸ which clearly indicates that a number

of species such as *Globigerinatella insueta* Cushman and Stainforth, *Globorotalia fohsi fohsi* Cushman and Ellisor, *G. fohsi lobata* Bermudez and *G. fohsi robusta* Bolli were restricted to the tropical belt in the Miocene, with cooler water in the higher latitudes such as the North Atlantic.

D. GRAHAM JENKINS

University of Canterbury,
New Zealand

- ¹ Cifelli, R. *Nature* 264, 431–432 (1976).
- ² Berggren, W. A. *Init. Rep. Deep Sea Drilling Project* 12, 965–975 (1972).
- ³ Jenkins, D. G. N. Z. J. *Geol. Geophys.* 8, 1088–1126 (1966).
- ⁴ Jenkins, D. G. N. Z. J. *Geol. Geophys.* 10, 1064–1078 (1967).
- ⁵ Bolli, H. M. U.S. Nat. Mus. Bull. 215, 97–123 (1957).
- ⁶ Blow, W. H. *Proc. First Int. Conf. plank. microfossils* (eds Bronniman, P. & Renz, H. H.) 1, 199–422 (1969).
- ⁷ Jenkins, D. G. *Micropaleontology* (in the press).
- ⁸ Jenkins, D. G. *Micropaleontology* 11, 265–277 (1965).

CIFELLI REPLIES—Clearly, the proposal that warm gyral water was greatly expanded in the North Atlantic during the Miocene needs further testing against the evidence of the fossil record. But in dismissing this generalisation as idle speculation, Jenkins seems to have lost sight of my arguments and has shown nothing incompatible with 'detailed biostratigraphy'. DSDP Sites 116, 117 were interpreted oceanographically as gyral margin, not tropics and there is no reason to expect an identity in composition of faunal zones. Plankton assemblages of North Atlantic gyral margins are characterised by mixed associations of cool high latitude and sub tropical species plus lesser numbers of tropical expatriates displaced to the north by the clockwise movement of the circulation¹. Mixed associations of this kind are well represented in DSDP Sites 116, 117, as discussed in my paper. If zonation of DSDP Sites 116, 117 reminds Jenkins of New Zealand zonation, then the oceanographic significance of the latter deserves reconsideration. That is, if faunal associations are mixed, as in DSDP Sites 116, 117 margins of warm gyral water may also be represented in the New Zealand Miocene.

Jenkins points out that a bore hole of Lower Miocene age near the Isles of Scilly lacks some 'warm water' species found in Trinidad. The species, however, that he lists are all extinct ones and the nature of the habitat they occupied is unknown. Actually, those species are either the same as, or closely related to, Oligocene species which are more analogous morphologically to modern cold water than warm water forms². They are a bad choice for warm water indicators.

Jenkins' claim that certain species, notably the *Globorotalia fohsi* group is restricted to the tropical belt is nullified by Bartlett³ who recorded

that species group and other tropical or subtropical forms from Nova Scotia.

R. CIFELLI

National Museum of
Natural History,
Washington, DC 20560

- ¹ Cifelli, R. & Benier, C. J. *Foram. Res.* 6, 259 (1976).
- ² Cifelli, R. *Syst. Zool.* 18, 154 (1969).
- ³ Bartlett, G. A. *Maritime Seds.* 4, 22 (1968).

U-shaped gene frequency distributions

GILPIN *et al.*¹ have recently questioned the usefulness of the frequency distribution of allele frequencies as a means of distinguishing between the neoclassical (neutralist) and balance (selectionist) interpretations of genic variation in natural populations. The conventional view has been that random genetic drift will generate a U-shaped distribution of allele frequencies at equilibrium, while all allele frequencies will be equally probable if they represent stable equilibria maintained by the superiority of heterozygotes. Gilpin *et al.* argue that the assumption of equiprobability is gratuitous, and that the frequency distribution of allele frequencies amongst overdominant loci will be determined by the joint probability distribution of the selection coefficients acting on the homozygotes. In particular, U-shaped frequency distributions are to be expected if the probability of obtaining a given selection coefficient falls off steeply with the magnitude of the coefficient. Such a distribution of selection coefficients seems plausible, and is in any case similar to that which is claimed to be the case by the neutralist school.

The result obtained by Gilpin *et al.* applies to overdominant loci, treated deterministically. It may be worth pointing out that a U-shaped equilibrium distribution of allele frequencies will also be generated deterministically amongst loci at which the heterozygote is intermediate in fitness. Consider a locus with two alleles, *A1* and *A2*, whose frequencies are *p* and *q* respectively. If the selection coefficient and the degree of dominance are given, the rate of substitution will vary directly with the genic variance, *pq*. Since this is maximal at *p=q=1/2*, and is minimal when either allele is near fixation, substitution will proceed rapidly at intermediate allele frequencies, but very slowly when either allele is very common. If we imagine an ensemble of loci behaving in this way, it is easy to see that most loci will accumulate in the regions where the rate of movement of allele frequency is lowest, that is near *p=1* and near *p=0*. In this way, the frequency distribution of allele frequencies at loci which are undergoing directional selec-

tion in an infinite population will be U-shaped at equilibrium.

GRAHAM BELL

Department of Biology,
McGill University,
Montreal, Quebec, Canada H3A 1B1

- ¹ Gilpin, E. A. *et al. Nature* 263, 497–499 (1976).

ONDRICEK *et al.* REPLY—Bell correctly notes a possibility we had not considered, namely that the commonly observed U-shaped allele frequency distribution is to be expected assuming that polymorphic loci are in the process of becoming fixed for a better allele; this is simply because the fastest rate for change of transient polymorphisms is at intermediate gene frequencies.

Two predictions follow from his model. The first is that the rate of evolution of a population is proportional to its level of heterozygosity. There are insufficient data to test this, but the common finding of high heterozygosities in physically stable environments (the deep sea^{2,3} and the tropics^{4,5}) is not auspicious. Nor is the occurrence of moderate levels of variation in a 'living fossil', *Limulus polyphemus*⁶, consistent with such a theory.

Second, the transient polymorphism model would predict that frequencies in the middle range, say 0.7 to 0.3, should not be conserved; that is, if related species or isolated populations of the same species share polymorphisms with allele frequencies in this range, it would argue for balancing selection. Tenacity for intermediate allele frequencies seems to be rather common. For example, the *Lacerta* data⁷ show that *L. melisellensis* retains two alleles at intermediate frequencies for phosphoglucumutase-2 on all islands larger than 0.05 km², and *L. sicula* shows the same pattern for esterase-4. Among four species of the *willistoni* group of *Drosophila*, the analogous pattern is observable for aldolase, xanthine dehydrogenase, and adenylate kinase-2 (ref. 4). The examples could be multiplied.

In summary, we think it likely that only a small fraction of polymorphic loci are transient, and in the process of becoming fixed for an overall 'better' allele.

ANATOLA ONDRICEK

E. A. GILPIN

M. E. SOULE

M. E. GILPIN

Biology Department
University of California, San Diego
La Jolla, California 92037

- ¹ Bell, G. *Nature* 268, 374 (1977).
- ² Schopf, T. J. M. & Gooch, G. L. *J. Geol.* 80, 481–483 (1972).
- ³ Ayala, F. J. & Valentine, J. W. *Marine Biol.* 27, 51–57 (1974).
- ⁴ Ayala, F. & Tracy, M. L. *Proc. natn. Acad. Sci. U.S.A.* 71, 999–1003 (1973).
- ⁵ Somero, G. N. & Soule, M. *Nature* 249, 670–672 (1974).
- ⁶ Selander, R. K., Yang, S. Y., Lewontin, R. C. & Johnson, W. E. *Evolution* 24, 402–414 (1970).
- ⁷ Gorman, G. C., Soule, M., Yang, S. Y. & Nevo, E. *Evolution* 29, 52–71 (1975).

reviews

Mayr's evolutionary mission

A. J. Cain

Evolution and the Diversity of Life: Selected Essays. By Ernst Mayr. Pp. ix+721. (Belknap/Harvard University: Cambridge, Massachusetts, 1976.) £15.

THIS book is most likely to be reviewed by those for whom it is not intended (myself included). At first sight it is a strange collection of scientific papers (not essays like Julian Huxley's), whole or more usually excerpted, classed under the headings: evolution, speciation, history of biology, philosophy of biology, theory of systematics, the species, man (merely a blast—well aimed at the time—against the excessive taxonomic splitting of fossil hominids), biogeography and behaviour. Some, such as Mayr's paper on change of genetic environment and evolution (1954), propounding a theory of genetic revolution in peripheral populations, are major contributions to evolutionary theory at a professional level; others, now translated into English, are elementary expositions of neo-Darwinian theory for the benefit of continental audiences.

Useful, indeed sometimes excellent, studies of important but neglected or misunderstood biologists (Lamarck, Louis Agassiz, Karl Jordan) are in the book, together with a ponderous (but correct) reply to Hennig's extraordinary ideas on evolutionary systematics, which could have been shortened, and tantalising snippets on topics as important as: accident or design (four pages); explanatory models in biology (five pages), and theory formation in developmental biology (six pages). The book is especially rich, as would be expected, in papers on speciation, the species and biogeography, although in the last only general discussions and conclusions are given, detailed documentation being left out. Throughout, papers have been adapted for this volume, but I am told that in every case where the author has changed his opinion this is duly noted between square brackets, so that the reader can see his intellectual progress. Mayr says himself in the general introduction that where he has included several essays on the same subject, this is done to "illuminate the maturation in my thinking". It would seem then that

there is something in the book for everyone, although perhaps not much for some.

I think this is true. Mayr has a better acquaintance with European continental evolutionary biology than many westerners, and is probably the best judge of what is useful among his papers for the continental readers. Anyone in Britain or the United States might be inclined to dismiss his two papers for continental audiences as not worth reprinting, but the appearance of Grassé's book, *L'Evolution du Vivant*, (1973) makes one pause. If this is the best that the doyen of French zoology can do, then a vast amount of elementary instruction is needed on the continent before one can even start discussion. Although he has acquired some reasonably up-to-date references, Grassé is still well within the tradition set by Flourens's notorious attack on the *Origin of Species*. Some of the papers in Mayr's book are in journals not easy to come by, and more people than European continentals, in particular undergraduates and pre-doctoral students, will find the collection extremely useful. What is there in it for others?

Any evolutionist or historian of biology will be interested to see how Mayr's thought has changed since 1940, but he will have to work out the changes for himself, and must refer to other papers not included in it. I am very grateful to Ernest Mayr for outlining to me the principal changes (the notes that follow are, however, mine). In 1942, following the theory accepted then, he thought of polymorphism as neutral, but in 1945 (in a paper in *Lloydia* not, alas, included in the book) he had already come to see that selection must be acting. Formerly he regarded evolution as basically a change in gene frequencies but in the 1970s as a change in organisms, that in gene-frequencies being merely a consequence of selection on organisms. Formerly, he took the view that evolution would go fastest and speciation would be most frequent in big populations, but now he thinks that small peripheral populations, that have undergone a genetic revolution when they have been established, are the principal source of new evolutionary forms.

Coordinate with this is his strong emphasis on the genotype not as a 'bean-bag' of genes but heavily co-adapted. In systematics, he points also to a sorting out of the ideas of the species as a taxon and as a category, previously confused in some of his earlier papers. In the highest levels of evolutionary theory, he is most impressed by the advent of population thinking, in which one is dealing with populations of interacting individuals, each of which is unique. It is clear from the book that he was profoundly impressed by a public encounter with Niels Bohr in which that great man seemed to misunderstand completely the greatest advance in biology. We have all met lesser men who enunciate such gems as "Molecular biology is biology, and all biology is molecular biology". Mayr has seen too much of the diversity of living things and the emergence of levels of organisation to be content with such a half-truth. He has always been well ahead in his appreciation of the complexities of natural selection, the enormous fluctuations in its rate, in short the biology of selection in the wild, although it took him some years (not documented here) to absorb the British work on the subject, and he apparently still puts faith in historic accident determining characters of great groups, and in the selective equivalence of isoalleles. This is not surprising as his own work has lain almost entirely in long-term evolution and the results rather than the action of selection.

This is a missionary book, against ignorance of the modern evolutionary synthesis, against the distortion of biology by molecular biologists, against the distortion of systematics by *a priori* considerations and against the neglect of major personalities in the development of evolutionism. Unfortunately, the style is not that of a missionary but a crusader, who believes that the only good heathen is a dead one. To be told that gradual evolution was accepted quite universally soon after 1859, that there is no such thing as mutation pressure (p 12) and yet that it has some direct results at the cellular level (p 111), is rather bludgeoning. Such remarks as "Thousands of experiments have proved that..." (no references), and "Discoveries in modern biology

indicate that..." (no references) are more reminiscent of political propaganda. The missionary may have spoken more truly than he knew in saying (p 34) "Merely asserting [these claims] dogmatically will not convince those who, until now, have been dis-

believers". It may even unsettle belief. What is the actual evidence not that genes interact but that genotypes are coadapted? □

A. J. Cain is Derby Professor of Zoology at the University of Liverpool, UK.

Plant transport processes

Transport in Plants. Vol. 3: Inter-cellular Interactions and Transport Processes. Edited by C. R. Stocking and U. Heber. Pp. xxii+517. (Springer: Berlin and New York, 1976.) DM145; \$59.50.

THIS is the third volume covering transport process in plants in the new series of the *Encyclopaedia of Plant Physiology*. The first two volumes covered phloem transport and transport in cells, tissues and organs. This third multi-author volume is concerned with intracellular interactions and transport processes, a topic of considerable current interest which received little coverage in the first series published 1955-58. Indeed, as the editors point out, this is an area which is still poorly understood, particularly in relation to plants. This last point is illustrated by a glance at the references cited in many of the chapters, a high proportion being concerned with animal or microbiol systems. Thus, although this volume should become outdated fairly rapidly, it will serve as a useful summary of progress to date and as an indicator for future research.

The book is divided into four major sections concerned with membrane structure, intracellular interactions, transport in relation to energy conservation, and the theory of membrane transport. There is a little overlap with the second volume in the series in the first and last of these sections as regards membrane structure and the principles of irreversible thermodynamics. This is largely unavoidable and, in general, this volume complements the others very well.

The first section containing one chapter on plant membranes is disappointing. For example, the few pages on membrane isolation are really too superficial for an encyclopaedia; a number of the subsections are covered by reference to one or two reviews of work on animals. The treatment of plant plasma membranes could also be misleading. Lists of characteristics and markers are given, although these are based on only a handful of papers and are the subject of considerable current debate.

The second section of eight chapters covers intracellular interactions involving the nucleus, chloroplasts, the organelles concerned with photorespiration, mitochondria, vacuoles, and the endomembrane system. The chloroplast is particularly well reviewed in three chapters concerning re-cycling, export and storage in this organelle, metabolite carriers, and compartmentation and transport in C_4 photosynthesis.

It is clear that, although we know a great deal about the structure and separate biochemistry of various individual organelles, our knowledge of their transfer processes and interactions is still in its infancy. This is illustrated by the discussion of the importance of inorganic pyrophosphate in maintaining maximal photosynthetic rates in intact, isolated chloroplasts; it is thought to work by regulating the movement of orthophosphate into the chloroplast. Although this process probably is completely artificial, it indicates the role of the cytoplasm in regulating photosynthetic rates *in vivo*.

The importance of the treatment of chloroplasts after isolation, often given less attention than the isolation procedure itself, is stressed and deserves much wider consideration. The chapter on vacuoles further emphasises the difficulty of studying organelles in isolation from the cytoplasm, although the digestive lysosomal-like role of the vacuoles now appears to be well established.

The third section of three chapters is concerned with ion transport and energy conservation and transfer in chloroplasts and mitochondria. Work on chloroplasts, in particular, provides strong support for the chemiosmotic interpretation in the great phosphorylation debate. The final section and chapter reviews the basic concepts used in the theoretical analysis of membrane transport processes.

The book is well illustrated and contains a thorough author and subject index. Although a number of the topics have been recently reviewed in other volumes, and in some cases by the same authors, they are here brought together in a single volume which, overall, lives up to the high standards set by the preceding numbers in this series.

J. L. Hall

J. L. Hall is Lecturer in Plant Physiology at the University of Sussex, UK.

Point defects

Point Defects in Crystals. By R. K. Watts. Pp. vi+132. (Wiley-Interscience; New York and London, 1977.) \$27.90; £16.50.

THIS monograph deals with the magnetic resonance and optical properties of point defects. The first six chapters are theoretical. They cover electronic states of defects with tightly bound electrons (rare earths, transition metals, the F centre), with loosely bound electrons (donors, acceptors, excitons), vibrational properties (localised modes, vibronic transitions, the Jahn-Teller effect), defect chemistry (equilibrium, thermodynamic potentials), theory of experimental methods (the spin Hamiltonian, optical properties), and electrons in covalent and ionic crystals. But this is not a textbook; supplementation by more pedagogic texts will be necessary. And the level is too advanced and too specialised for undergraduates, though suitable for post-graduates.

The next four chapters are reviews of point defects in the group IV, III-V, II-VI and alkali halide crystals. Materials dealt with include Si, Ge, diamond, GaP and GaAs. The II-VIs and alkali halides are dealt with by considering the defects and their manifestation in the different compounds, rather than by taking each compound individually. The coverage is thorough, although the author does not claim that it is exhaustive. The latest references are 1974 to 1976, depending on the material.

Reviews are, however, sooner or later overtaken by events, and this has already happened in at least one material Dr Watts has covered—diamond. This illustrates a problem inherent in reviewing fields of research other than one's own. (From the references it seems that Dr Watts's subject is the II-VI compounds.) Although the picture he presents of defects in diamond is largely correct, it is incomplete. And the identification of one important defect, though generally accepted before 1972, is widely disbelieved now. A diamond specialist would be aware of all this. One wonders if any of the other chapters have the same deficiency.

Nonetheless, the book is a useful introduction to the spectroscopic properties of these materials, provided it is supplemented by attention to the current literature and to more specialised reviews.

John Walker

John Walker is a Royal Society European Programme Research Fellow studying point defects and ion implantation in diamond with the Groupe de Physique des Solides de l'Ecole Normale Supérieure, Université Paris VII.

Volcanoes and eruptions

Volcanoes of the Earth. (Revised edition.) By Fred M. Bullard. Pp. 579. (University of Texas Press: Austin and London, 1976.) £20.

Volcanoes of the Earth is, as the dust jacket blurb states, a revised and enlarged edition of Bullard's *Volcanoes: In History, In Theory, In Eruption*, first published in 1962. It is a handsome volume, very satisfying to leaf through and to have on one's shelves. In contrast to the numerous cheaply produced textbooks that one encounters these days, this one gives the impression of being a *real* book, and one which the publishers seem to have spared no expense in producing. There are, of course, numerous text figures and photographs, most of them excellent, and even a section of colour photographs. Most striking, however, is the liberal use of white paper. Many diagrams have a whole page to themselves, and the text pages have six centimetre margins.

There are three main parts to the text. The first deals with the background to volcanology, the second covers the types of eruptions, and the third is rather a hotch-potch entitled "Theory, Cycles, Utilization and Environmental Effects of Volcanoes".

The core of the book is the section on types of volcanic eruptions, and it is here that its major strengths and weaknesses lie. Almost all of the useful parts of the book are concentrated in this section; detailed histories of individual volcanoes and eruptions. Bullard exhibits the evidence of much patient scholarship here. He has obviously tracked down and read a vast number of original references, including some very obscure ones. To take just one example, his account of the eruption of Mt Katmai in 1912 and the subsequent scientific investigations is unquestionably better than any other book in print. Many other authors have written about the same events, but they have based their accounts on secondary or even tertiary sources, thus blurring and muddying the facts. Having gone right back to the primary sources, Bullard's descriptions have a notable freshness and precision. Neither are they mere dry reviews. Bullard uses his narrative skill to present his material in a thoroughly readable, stimulating style.

Having said this, it is unfortunate that one has to look at the other, tarnished side of the coin. Bullard nowhere discusses adequately the criteria by which he recognises different types of eruption, and often seems to confuse a *type* of eruption with an individual

volcano. Thus, chapter 9 on "The Strombolian Type of Volcanic Eruption" makes no mention of *any* volcano other than Stromboli. (About one-third of the chapter describes a visit made by Bullard and his wife to the island.) Similarly, chapter 10 on "The Hawaiian Type of Volcanic Eruption" deals exclusively with eruptions in the Hawaiian islands. In chapter 9, Bullard does at least begin with a few vague comments on the nature of Strombolian eruptions; in chapter 10, he prefers to discuss Captain Cook and the early history of the islands.

The book would have been strengthened by at least some discussion of modern work on classification of volcanic eruptions by granulometric analysis of pyroclastic material. This omission is characteristic of the text as a whole. The dust jacket proclaims that "Recent advances in heat flow studies and the concept of sea floor spreading and plate tectonics are incorporated to present a view of volcanology consistent with our present state of knowledge". This is emphatically not the

case. In fact, the book has generally a somewhat dated atmosphere to it. Only a small proportion of the many works referenced were published in this decade. There are, indeed, two chapters on island arcs and plate tectonics, but they sit rather uncomfortably at the end of the book as rather unnecessary appendages, not well integrated with the rest of the text. They are competent enough reviews, but are unlikely to be of much value at present, when there are so many other good reviews around, and particularly because Bullard dampens any enthusiasm his readers may have for plate tectonics by implying quite clearly that he is not terribly impressed by it all.

In summary, *Volcanoes of the Earth* is an excellent source of facts on historical volcanic eruptions. As a review of modern volcanology it leaves much to be desired.

Peter Francis

Peter Francis is Lecturer in Earth Sciences at The Open University, Milton Keynes, UK.

Crystalline rocks

Evolution of the Crystalline Rocks. Edited by D. K. Bailey and R. MacDonald. Pp. ix+484. (Academic: London and New York, 1976.) £16; \$35.

ALMOST half a century has elapsed since the appearance of Bowen's *Evolution of the Igneous Rocks* (1928). In that time major advances in both equipment and experimental techniques have led to a wealth of published experimental data, presenting the contributors with an unenviable task in writing a sequel to Bowen's work, within the confines of a single volume. Not surprisingly all aspects of the contribution of experimental petrology to the understanding of the evolution of crystalline rocks could not be covered, but the omissions in the igneous section, noticeably the basic and intermediate rocks, have led to a marked imbalance in the text.

The opening chapter on experimental methods offers a basic introduction for newcomers to experimental petrology. More detailed accounts of specific techniques can be found in the references cited. The choice of PT subdivisions in the metamorphic section has inevitably led to a certain amount of duplication. This has, however, resulted in some interesting insights into the differing criteria used by individual contributors in assessing the various conflicting sets of data (see Greenwood and Schreyer on the stability of Al₂SiO₅ polymorphs).

Newton and Fyfe give a comprehen-

sive account of blueschist metamorphism (57 pages), but have rather scantily covered eclogites and granulites (10 pages). Greenwood offers one of the best sections, striking a very good balance between the major aspects of experimental petrology and its application to quantifying the intensive parameters in a range of metamorphic rocks. Schreyer has adopted a slightly different approach concentrating more on the phase chemistry aspects at high temperatures and low pressures.

The section on granitic rocks is mainly concerned with the crystallisation of haplo granitic melts. The reader's attention is drawn to the paucity of data for water undersaturated systems and the composition of the vapour phase in equilibrium with solidus reactions. In his concluding remarks, Luth has essentially critically reviewed his own contribution, by pointing out the omissions in dealing with the ultimate source of granitic melts and the volcanic rocks of granitic composition. Bailey presents a very concise review of the major aspects of alkaline rocks, delineating some of the problems in the genesis and subsequent crystallisation of the melts.

The imbalance in some of the contributions and the questionable policy of omitting certain major aspects of igneous experimental petrology will probably reduce the usefulness of this book as an undergraduate text.

J. Nolan

J. Nolan is Lecturer in Petrology in the Geology Department, Imperial College, London, UK.

Atomic and molecular processes

Atomic Processes and Applications. (In Honour of David R. Bates' 60th Birthday.) Edited by P. G. Burke and B. L. Moiseiwitsch. Pp. x+533. (North-Holland: Amsterdam, New York and Oxford, 1976.) Dfl.165; \$65.95.

THIS book forms a remarkable tribute to the scientific work in atomic and molecular physics over the past 40 years of David R. Bates' life. The scene is set in a perceptive and affectionate tribute to David Bates on his sixtieth birthday by Sir Harrie Massey, who begins by recalling his first meeting with Bates when, in 1934, he went to Queen's University, Belfast, as Lecturer in Mathematical Physics. Bates was one of the group of able young men from Ulster attracted by Massey to work in theoretical physics. The work based on their cooperation first at Belfast, then at University College, London, and later, also, in the school founded by David Bates when he returned as Professor to Queen's University in 1951, represents a large part of the progress made over this period in the study of atomic and molecular processes. It is indeed a striking example of the influence of the great leader and teacher in science that so many of the authors of articles in this book have been students at Queen's University. All of them have more or less close connections with either University College, London or Queen's. In this respect, it is not surprising that the book conveys an extraordinary sense of family.

Apart from the introductory tribute by Sir Harrie Massey, the thirteen articles which make up the book form a highly readable account of the present state of progress in the study of atomic processes. The first three articles deal with the Earth's atmosphere: M. Nicolet on Stratospheric Aeronomy, J. C. G. Walker on the Upper Atmosphere, and M. B. McElroy on Problems in Atmospheric Pollution. M. Nicolet considers the principal reactions first in an oxygen atmosphere, then in turn adding hydrogen, nitrogen and halogens. The theoretical problem of stratospheric ozone is resolved and the situation concerning minor constituents described. J. C. G. Walker provides an admirable summary of present knowledge of the chemistry of the E and F regions, stressing the importance in the understanding of the phenomena of dissociative recombination of mole-

cular ions as the process for removing ions and electrons, as first suggested by Bates and Massey in 1947. M. B. McElroy, in an excellent account of man's impact on the global environment, pays tribute to the classic contribution of Bates and Witherspoon (1952).

The theme of molecules in interstellar space is taken up by A. Dalgarno, who deals with formation and destruction mechanisms for CH and CH⁺. The problem posed for the abundance of CH⁺ by the existence of molecular hydrogen in interstellar clouds, is considered. The process of di-electronic recombination of special interest in connection with the solar corona, is the subject of a theoretically interesting treatment by M. J. Seaton and P. J. Storey.

The remaining eight chapters of the book are devoted to an authoritative series of reviews of some of the main fields of study in atomic and molecular processes. Pioneering contributions were made in many of these by David Bates, and he has continued to contribute to them up to the present day. Photoionisation of atomic systems and electron scattering by atoms are the subject of masterly reviews by P. G. Burke and M. C. McDowell. B. L. Moiseiwitsch and A. L. Stewart contribute chapters, respectively, on negative ions and oscillator strengths. There is a thought-provoking chapter on computational methods by I. C. Percival.

The book concludes with three chapters on ionic recombination and atomic collision processes by M. R. Flannery, R. McCarroll, K. L. Bell and A. E. Kingston. Throughout this series of chapters, it is fascinating to follow the increased understanding which has come from the use of atomic models of increasing sophistication and from the growth of computational facilities. Another interesting aspect is the balance of success in appropriate régimes of quantal semi-classical and classical methods.

The editors are to be congratulated on the volume of work which they have brought together. The presentation is of uniform clarity and free, as far as I could detect, from all but the most trivial misprints. It must be a great joy to Professor Bates to have gathered together for him by this group of his students, colleagues and friends a description of a scientific edifice which he helped to found and did so much to build. Apart from this celebratory aspect the book is to be recommended as providing an admirable description of the state of the art in the study of atomic processes, which are of such increasing importance in a growing number of fields of interest in contemporary physics.

J. C. Gunn

J. C. Gunn is Professor of Natural Philosophy at the University of Glasgow, Scotland, UK.

Earth pictures

Mission to Earth: Landsat Views the World. By N. M. Short, P. D. Lowman, Jr, S. C. Freden and W. A. Finch, Jr. (NASA: Washington, DC, 1976.) \$14.

LANDSAT, launched in 1972, was the first of a generation of unmanned satellites dedicated to repetitive surveillance of the continents and adjacent coastal waters to gather data for a variety of practical applications in Earth Resources disciplines, such as cartography, geological mapping, mineral resource evaluation, water inventory, coast and wetlands management, land-use assessment, and so on.

The above publication contains some 400 colour plates of outstanding Landsat pictures of areas all over the World, compiled and selected from more than 100,000 processed and reconstructed images, obtained during its first four years of operation. Each plate is accompanied by a carefully prepared narrative caption, summarising notable

features. Taking into account that these photographs were available anyway from their Earth Resources program, it can only be warmly welcomed that NASA has made this selection of outstanding pictures available to the general public at the comparatively low price of \$14. These photographs are likely to be of great interest not only to the various resource specialists amongst the appropriate Government authorities in the various countries concerned, but also to the serious teachers and students in geography, geology and allied disciplines, and to the general public with an interest in travel and geography.

Copies and slides of individual photographs are also obtainable, as well as free copies of an educational supplement, the *Educator's Guide*, which provides a general description of the Landsat program, the operation of the satellite and an explanation of remote sensing and imagery with suggested classroom uses of the material.

P. Schagen

P. Schagen works in the Applied Physics Division at the Philips Research Laboratories, Surrey, UK.

Recent scientific and technical books

Chemistry

- KONIG, E., and KREMER, S. *Ligand Field Energy Diagrams*. Pp.viii+454. ISBN-0-306-30946-7. (New York and London: Plenum Press, 1977.) \$59.40.
- KRUUS, Peeter. *Liquids and Solutions: Structure and Dynamics*. Pp.x+582. ISBN-0-8247-6427-7. (New York and Basel: Marcel Dekker, Inc., 1977.) \$Fr.145.
- LADD, M. F. C., and PLAMER, R. A. *Structure Determination by X-ray Crystallography*. Pp.xvi+393. ISBN-0-306-30844-4. (New York and London: Plenum Press, 1977.) \$35.40.
- LEE, Lieng-Huang (edited by). *Characterization of Metal and Polymer Surfaces*. Vol. 1: Metal Surfaces. Pp.xiv+517. ISBN-0-12-442101-6. \$26.50; £18.80. Vol. 2: Polymer Surfaces. Pp.xv+465. ISBN-0-12-442102-4. \$24; £17.05. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.)
- LENK, R. *Brownian Motion and Spin Relaxation*. Pp. x+250. ISBN-0-444-41592-0. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl.110; \$44.90.
- LEWIS, D. A., and CHARNOCK, P. (compiled by). *Index of Reviews in Organic Chemistry: Second Cumulative Volume*. 1976. Pp.308. ISBN-0-85186-539-9. (London: The Chemical Society, 1977.) £6.50; \$13.
- LEVY, F. (edited by). *Structural Chemistry of Layer-Type Phases. (Physics and Chemistry of Materials with Layered Structures)*. Pp.viii+392. ISBN-90-277-0714-6. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl.105; \$39.50.
- McAULIFFE, C. A. (edited by). *The Chemistry of Mercury. (Aspects of Inorganic Chemistry)*. Pp.vii+288. ISBN-0-333-17863-7. (London and Basingstoke: The Macmillan Press, Ltd., 1977.) £25.
- MARCHAND, Alan P., and LEHR, Roland, E. (edited by). *Pericyclic Reactions*, Vol. 1. (Organic Chemistry: a Series of Monographs, Vol. 35.) Pp.xi+286. ISBN-0-12-470501-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) np.
- MARCUS, Y., and BEN-DOR, L. (congress editors). *Plenary lectures presented at the 25th International Congress of Pure and Applied Chemistry, Jerusalem, Israel, 6-11 July 1975. (International Union of Pure and Applied Chemistry, in conjunction with Israel Academy of Science and Humanities, National Council for Research and Development, and Israel Chemical Society.)* Pp.73. ISBN-0-08-020952-1. (Oxford and New York: Pergamon Press, 1977.) £10.50.
- MILLER, William H. (edited by). *Dynamics of Molecular Collisions, Part B. (Modern Theoretical Chemistry, Vol. 2)*. Pp.xv+380. ISBN-0-306-33502-6. (New York and London: Plenum Press, 1976.) \$39.50.
- MILLICH, Frank, and CARRAHER, Jr, Charles E. (edited by). *Interfacial Synthesis*. Vol. 1: Fundamentals. Pp.xi+298. ISBN-0-8247-6372-6. (New York and Basel: Marcel Dekker, Inc., 1977.) \$Fr.113.
- NECKERS, Douglas C., and DOYLE, Michael P. *PSI Study Guide for Organic Chemistry*. Prepared by Erich C. Blosser. Pp.xxii+246. ISBN-0-471-08345-3. (New York and London: John Wiley and Sons, Inc., 1977.) \$6.85; £4.
- NECKERS, Douglas C., and DOYLE, Michael P. *Organic Chemistry*. Pp.xxxvi+1147. ISBN-0-471-63091-8. (New York and London: John Wiley and Sons, 1977.) £16.50; \$27.90.
- NEWTON, G. W. A. (senior reporter). *Radiochemistry*. Vol. 3. (A Review of the Literature Published during 1974 and 1975. A Specialist Periodical Report.) Pp.viii+141. ISBN-0-85186-274-8. (London: The Chemical Society, 1976. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts; American Chemical Society, 1155 Sixteenth Street, N.W., Washington, DC.) £11.50; \$23.
- PARISH, R. V. *The Metallic Elements*. Pp.xii+254. ISBN-0-582-44278-8. (London and New York: Longman, 1977.) £7.95.
- PARKER, W. (senior reporter). *Allylic Chemistry*. Vol. 5. (A Review of the Literature Published during 1975. A Specialist Periodical Report.) Pp. ix+439. ISBN-0-85186-612-3. (London: The Chemical Society, 1977. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts; American Chemical Society, 1155 Sixteenth Street, N.W., Washington, DC.) £28; \$56.
- PATAI, Saul (edited by). *The Chemistry of Double-Bonded Functional Groups*. Part 1: Pp. xiv+1-651. ISBN-0-471-99464-2. £29; \$52.50. Part 2: Pp.xv+653-1343. ISBN-0-471-99465-0. £29.50; \$55. (The Chemistry of Functional Groups, Supplement A.) (London and New York: John Wiley and Sons, 1977.) Supplement A, Parts 1 and 2 \$58.50; \$107.50.
- PORTER, Roger S., and JOHNSON, Julian, F. (edited by). *Analytical Calorimetry*, Vol. 4. Pp.ix+251. ISBN-0-306-35244-2. (New York and London: Plenum Press, 1977.) \$33.
- PULLMAN, Bernard, and GOLDBLUM, Natan (edited by). *Metal-Ligand Interactions in Organic Chemistry and Biochemistry, Part I. (Proceedings of the Ninth Jerusalem Symposium on Quantum Chemistry and Biochemistry held in Jerusalem, March 29th-April 2nd, 1976.) (The Jerusalem Symposia on Quantum Chemistry and Biochemistry, Vol. 9.)* Pp.x+396. ISBN-90-277-0751-0. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl.100; \$39.50.
- ROBIN, J. P. (colloquium editor). *Plenary Lectures Presented at the XVIII Colloquium Spectroscopicum International, Grenoble, France, 15-19 September 1975.* (International Union of Pure and Applied Chemistry (Physical Chemistry Division), in conjunction with Groupement pour l'Avancement des Méthodes Spectroscopiques et Physico-Chimiques d'Analyse (GAMS).) Pp.45-126. ISBN-0-08-021569-6. (Oxford and New York: Pergamon Press, 1976.) £4.45.
- ROMANOV, A. (conference editor). *Modified Polymers, their Preparation and Properties. (Main Lectures presented at the Fourth Bratislava Conference on Polymers, Bratislava, Czechoslovakia, 1-4 July 1975.) (International Union of Pure and Applied Chemistry (Macromolecular Division), in conjunction with Slovak Academy of Sciences, Slovak Chemical Society, and the Slovak Technical University.)* Pp.70. ISBN-0-08-020953-X. (Oxford and New York: Pergamon Press, 1977.) £7.50.
- ROSENBERG, Robert M. *Principles of Physical Chemistry*. Pp.xix+745. (Oxford and New York: Oxford University Press, 1977.) £15.75 net.
- ROUQUEROL, J., and SABBAGH, R. (conference editor). *Chemical Thermodynamics—4. (Plenary lectures presented at the Fourth International Conference on Chemical Thermodynamics, Université des Sciences et Techniques de Languedoc, Montpellier, France, 26-30 August 1975.) (International Union of Pure and Applied Chemistry, Physical Chemistry Division, in conjunction with Société Chimique de France, Société Française de Chimie Physique and Société Française Thermiciens.)* Pp.245-331. ISBN-0-08-021366-9. (Oxford and New York: Pergamon Press, 1976.) £8.90.
- SAWYER, Donald T. (edited by). *Electrochemical Studies of Biological Systems. (ACS Symposium Series, No. 38.)* Pp.viii+216. ISBN-0-8412-0361-X. (Washington, DC: American Chemical Society, 1977.) \$15.50.
- SEGAL, Gerald A. (edited by). *Semiempirical Methods of Electronic Structure Calculation. Part A: Techniques. (Modern Theoretical Chemistry, Vol. 8.)* Pp.xvii+274. ISBN-0-306-33507-7(v.7). (New York and London: Plenum Press, 1977.) \$47.40.
- SEGAL, Gerald A. (edited by). *Semiempirical Methods of Electronic Structure Calculation. Part B: Applications. (Modern Theoretical Chemistry, Vol. 8.)* Pp.xvii+308. ISBN-0-306-33508-5(v.8). (New York and London: Plenum Press, 1977.) \$47.40.
- STEVENS, John G., and STEVENS, Virginia E. (edited by). *Mössbauer Effect Data Index Covering the 1975 Literature*. Pp.x+445. ISBN-0-306-65146-7. (New York and London: IFI/Plenum, 1976.)

THOMSON, R. H. (symposium editor). *International Symposium on Marine Natural Products. (Plenary lectures presented at the International Symposium on Marine Natural Products, Aberdeen, Scotland, 8-11 September 1975.) (International Union of Pure and Applied Chemistry, Organic Chemistry Division, in conjunction with The Chemical Society, Perkin Division.)* Pp.44. ISBN-0-08-021242-5. (Oxford and New York: Pergamon Press, 1977.) £4.45.

TRIPPETT, S. (senior reporter). *Organophosphorus Chemistry, Vol. 8. (A Review of the Literature published between July 1975 and June 1976. A Specialist Periodical Report.)* Pp.xi+289. ISBN-0-85186-076-1. (London: The Chemical Society, 1977. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts; American Chemical Society, 1155 Sixteenth Street, N.W., Washington, DC.) £22; \$44.

VINCENT, Alan. *Molecular Symmetry and Group Theory: a Programmed Introduction to Chemical Applications*. Pp. 156. ISBN-0-471-01867-8. (London and New York: John Wiley and Sons, 1977.) £5.90; \$12.

WEEDON, B. C. L. (symposium editor). *Carotenoids—4. (Main lectures presented at the Fourth International Symposium on Carotenoids, Berne, Switzerland, 25-29 August 1975.) (International Union of Pure and Applied Chemistry, Organic Chemistry Division, in conjunction with Swiss Science Council, Comité Suisse de Chimie, and Swiss Chemical Society.)* Pp.97-243. ISBN-0-08-020974-2. (Oxford and New York: Pergamon Press, 1977.) £16.65.

WELLS, A. F. *Three-Dimensional Nets and Polyhedra. (Wiley Monographs in Crystallography.)* Pp.xii+268. ISBN-0-471-02151-2. (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) £22; \$36.

YEN, T. F. (edited by). *Chemistry of Marine Sediments*. Pp.vi+265. ISBN-0-250-40103-7. (Ann Arbor: Ann Arbor Science Publishers, Inc.; Chichester: John Wiley and Sons, Ltd., 1977.) £10.75; \$21.

ZILBERSHTEIN, Kh. I. (edited by). *Spectrochemical Analysis of Pure Substances*. Translated by J. H. Dixon. Pp. 435. ISBN-0-85274-264-9. (Bristol: Adam Hilger, Ltd., 1977.) £27 net.

Technology

ANDERSON, Garron P., BENNETT, S. John, and DeVRIES, K. Lawrence. *Analysis and Testing of Adhesive Bonds*. Pp.xviii+255. ISBN-0-12-056550-1. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$21; £14.90.

BAKER, L. R. (edited by). *British Electro-Optics. (Proceedings of a Conference at the British Export Marketing Centre, Tokyo, in December, 1975.)* Pp.vii+139. ISBN-0-85066-101-3. (London: Taylor and Francis, Ltd., 1977.) £8.50 net.

BLAINE, J. C. D. *The End of an Era in Space Exploration: From International Rivalry to International Cooperation*. Pp.xv+199. ISBN-87703-080-4. (Science and Technology Series, Vol. 42.) (San Diego, Calif.: American Astronautical Society, 1976. Distributed by Univelt, P.O. Box 28130, San Diego.) n.p.

COLUCCI, Joseph M., and GALLOPOULOS, Nicholas E. (edited by). *Future Automotive Fuels: Prospects, Performance, Perspective*. Pp.ix+380. ISBN-0-306-31017-1. (New York and London: Plenum Press, 1977.) \$47.40.

COULSON, J. M., and RICHARDSON, J. F. *Chemical Engineering*. Vol. 1: *Fluid Flow, Heat Transfer and Mass Transfer*. With editorial assistance from J. R. Backhurst and J. H. Harker. Third edition (SI Units). Pp.xiii+449. ISBN-0-08-021015-5. (Oxford and New York: Pergamon Press, 1977.) Hardcover £10.50; \$25. Flexi cover £7.50; \$15.

COURTOIS, P. J. *Decomposability: Queueing and Computer System Applications. (Association for Computing Machinery, Inc. Monograph Series.)* Pp.xiii+201. ISBN-0-12-193750-X. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$17.50; £12.40.

ELCOCK, E. W., and MICHIE, Donald (edited by). *Machine Intelligence 8*. Pp.630. ISBN-85312-058-7. (Chichester: Ellis Horwood, Ltd.; New York: Halstead Press, a Division of John Wiley and Sons, Inc., 1977.) £24; \$45.60.

FLURSCHEIM, Charles H. *Engineering Design Interface Management Philosophy*. Pp.138. ISBN-0-85072-051-6. (London: Design Council Publications, 28 Haymarket, SW1, 1977.) £4.95 net.

FREY, Peter W. (edited by). *Chess Skill in Man and Machine. (Texts and Monographs in Computer Science.)* Pp.xi+217. ISBN-3-540-07957-2. (Berlin and New York: Springer-Verlag, 1977.) DM.36.20; \$16.

FRIEDLANDER, S. K. *Smoke, Dust and Haze: Fundamentals of Aerosol Behavior*. Pp.xvii+317. ISBN-0-471-01468-0. (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) \$19.50; £11.35.

HARTNETT, James P., and IRVINE, J. Thomas F. (edited by). *Advances in Heat Transfer*, Vol. 13. Pp.xiii+280. ISBN-0-12-020013-9. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$30; £21.30.

HOLMES, R. *The Characteristics of Mechanical Engineering Systems. (Pergamon International Library of Science, Technology, Engineering and Social Studies.)* Pp.viii+157. ISBN-0-08-021032-5. (Oxford and New York: Pergamon Press, 1977.) n.p.

JAEGER, Charles. *Fluid Transients in Hydro-Electric Engineering Practice*. Pp.xvi+413. ISBN-0-216-90225-8. (Glasgow and London: Blackie, 1977.) £18.50.

KNIGHT, J. (edited by). *Operation of Instruments in Adverse Environments, 1976. (Invited and contributed papers from the Conference held in London on 4 and 5 October 1976.)* Pp.134. (Conference Series No. 34.) ISBN-0-85498-125-X. (Bristol and London: The Institute of Physics, 1977. Distributed by Physics Trust Publications, Blackhorse Road, Letchworth, Herts; American Institute of Physics, 335 East 45 Street, New York.) £16; \$27.

LAITHWAITE, E. R. (edited by). *Transport Without Wheels*. Pp.ix+322. ISBN-0-236-40066-5. (London: Paul Elek (Scientific Books), Ltd., 1977.) £15.

LEONDES, C. T. (edited by). *Control and Dynamic Systems: Advances in Theory and Applications*, Vol. 13. Pp.xviii+365. ISBN-0-12-012713-X. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$25.50; £18.10.

McCALL, James L., and FRENCH, P. M. (edited by). *Interpretive Techniques for Microstructural Analysis*. Pp.vii+201. ISBN-0-306-31036-8. (New York and London: Plenum Press, 1977.) \$30.

McVEIGH, J. C. *Sun Power: an Introduction to the Applications of Solar Energy. (Pergamon International Library of Science, Technology, Engineering and Social Studies.)* Pp.x+208. ISBN-0-08-020862-2. (Oxford and New York: Pergamon Press, 1977.) Hardback £6; Flexi £2.75.

MICROPROCESSOR SYSTEMS: DEVELOPMENT AND APPLICATION. Pp.vi+90. ISBN-0-902852-75-2. (Guildford, Surrey: IPC Science and Technology Press, 1977. Published in association with the journal *Microprocessors*.) £7; \$15.50.

MILORA, Stanley L., and TESTER, Jefferson W. *Geothermal Energy as a Source of Electric Power: Thermodynamic and Economic Design Criteria*. Pp.186. ISBN-0-262-13123-4. (Cambridge, Mass. and London: The MIT Press, 1977.) £10.50.

MORGENTHAUER, George W., and SILVER, Aaron N. (edited by). *Energy Delta: Supply vs. Demand. (Science and Technology Series, Vol. 35.)* Pp.xv+590. ISBN-87703-082-0. (San Diego, Calif.: American Astronautical Society, 1976. Distributed by Univelt, Inc., P.O. Box 28130, San Diego.) \$40.

MORRIS, David J. *Introduction to Communication Command and Control Systems*. Pp.ix+308. ISBN-0-08-020378-7. (Oxford and New York: Pergamon Press, 1977.) £15.

PACEY, Arnold (compiled and edited by). *Water for the Thousand Millions. (Written by the Water Panel of the Intermediate Technology Development Group founded by Dr E. F. Schumacher.)* Pp.vi+59. ISBN-0-08-021805-9. (Oxford and New York: Pergamon Press, 1977.) n.p.

PAICE, C. D. Information Retrieval and the Computer. Pp.206. ISBN-0-354-04095-2. (London: Macdonald and Jane's, 1977.) £5.95.

REAY, David A. Industrial Energy Conservation: a Handbook for Engineers and Managers. Pp.xii+358. ISBN-0-08-021716-8. (Oxford and New York: Pergamon Press, 1977.) n.p.

ROBERTS, A. Geotechnology: An Introductory Text for Students and Engineers. Pp.xvii+347. ISBN-0-08-021594-7. (Oxford and New York: Pergamon Press, 1977.) Hard cover £19.50; Flexi cover £9.75.

ROSE, J. W., and COOPER, J. R. (edited by). Technical Data on Fuel. Seventh edition. Pp.xi+343. ISBN-0-7073-0129-7. (London: The British National Committee, World Energy Conference, 34 St. James's Street, SW1, 1977. Distributed by Scottish Academic Press, 33, Montgomery Street, Edinburgh, EH7 5JX, Scotland.) £30; £75.

ROSS, Monte (edited by). Laser Applications, Vol. 3. Pp.xi+238. ISBN-0-12-431903-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £23; £16.35.

SALT, Brian, G. D. Basic Animation Stand Techniques. Pp.239. ISBN-0-08-021368-5. (Oxford and New York: Pergamon Press, 1977.) £6.25.

SCHURING, Dieterich J. Scale Models in Engineering: Fundamentals and Applications. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xii+299. ISBN-0-08-020806-6. (Oxford and New York: Pergamon Press, 1977.) Hard cover £10; Flexi £7.

SHUVAL, Hillel I. (edited by). Water Renovation and Reuse. Pp.xiii+463. ISBN-0-12-641250-2. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £29.50; £20.95.

STEINHOFF, Ernst A. (edited by). The Eagle Has Returned. (Science and Technology, Vol. 43.) Pp.xvi+355. ISBN-87703-081-2. (San Diego, Calif.: American Astronautical Society, 1976. Distributed by Univelt, Inc., P.O. Box 28130, San Diego.) £30.

STERN, Arthur C. (edited by). Air Pollution. Vol. 4: Engineering Control of Air Pollution. Third edition. (Environmental Sciences: An Interdisciplinary Monograph Series.) Pp.xvii+946. ISBN-0-12-666604-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £49.50; £35.15.

TACCONI, G. (edited by). Aspects of Signal Processing, with Emphasis on Underwater Acoustics. (Proceedings of the NATO Advanced Study Institute held at Portovenere, La Spezia, Italy, 30 August—11 September 1976.) Part 1: Pp.xvii+1400. Part 2: Pp.xi+401-787. (NATO Advanced Study Institutes Series. Series C: Mathematical and Physical Sciences, Vol. 33.) ISBN-90-277-0798-7. (Dordrecht-Holland and Boston, Mass.: D. Reidel Publishing Company, 1977. Published in Co-operation with NATO Scientific Affairs Division.) Dfl.165; \$66.

TIMMERHAUS, K. D., REED, R. P., and CLARK, A. F. (edited by). Advances in Cryogenic Engineering, Vol. 22. Pp.xi+559. ISBN-0-306-38022-6. (New York and London: Plenum Press, 1977.) \$51.

TOULOUKIAN, Y. S., and MAKITA, Tadashi. Specific Heat: Nonmetallic Liquids and Gases. (Thermophysical Properties of Matter, Supplement to Volume 6.) Pp.xi+157. ISBN-0-306-67091-1. (New York and Washington: IFI/Plenum, 1976.) \$35.40.

Earth Sciences

ANTHONY, John W., WILLIAMS, Sidney A., and BIDEAUX, Richard A. Mineralogy of Arizona. Pp.xvii+252. ISBN-0-8165-0471-7. (Tucson, Arizona: The University of Arizona Press, 1977.) Cloth \$22.50; Paper \$9.75.

BULLARD, Fred M. Volcanoes of the Earth. Revised edition. Pp.579. ISBN-0-292-78701-4. (Austin and London: University of Texas Press, 1976.) £20.25.

CLARK, John R. Coastal Ecosystem Management: a Technical Manual for the Conservation of Coastal Zone Resources. (The Conservation Foundation.) Pp.x+928. ISBN-0-471-15854-2. (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) £28.90; \$48.50.

COOK, T. D., and BALLY, A. W. (edited by). Stratigraphic Atlas of North and Central America. Prepared by the Exploration Department of Shell Oil Company, Houston, Texas. Pp.272. ISBN-0-691-08189-1. (Princeton, NJ: Princeton University Press, 1977.) Cloth £37.40; Spiral bound £11.30.

FRASER, Donald G. (edited by). Thermodynamics in Geology. (Proceedings of the NATO Advanced Study Institute held in Oxford, England, September 17-27, 1976.) (NATO Advanced Study Institutes Series. Series C: Mathematical and Physical Sciences.) Pp.x+410. ISBN-90-277-0794-4. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl.90; \$36.50.

GRAY, J. M., and LOWE, J. J. (edited by). Studies in the Scottish Lateglacial Environment. Pp.xiii+197. ISBN-0-08-020498-8. (Oxford and New York: Pergamon Press, 1977.) £7.95.

GODARD, Alain. Pays et Paysages du Granite: Introduction à Une Géographie des Domaines Granitiques. (Le Géographe) Pp.232. (Paris: Presses Universitaires de France, 1977.) n.p.

GRIBBIN, John. Our Changing Planet. Pp.165. ISBN-0-7045-0247-X. (London: Wildwood House, Ltd., 1977.) £5.95 net.

GUILLAUME, André. Analyse des Variables Regionalisées: Traitement du Signal en Sciences de la Terra. Pp.vi+180. ISBN-2-901172-01-6. (Paris: Doin Editeurs, 1977.) 130 francs.

JAKUCS, Laszlo. Morphogenetics of Karst Regions: Variants of Karst Evolution. Pp.284. ISBN-0-85274-309-2. (Bristol: Adam Hilger; Budapest: Akademiai Kiadó, 1977.) £17 net.

JOHNSON, Arvid M. Styles of Folding: Mechanics and Mechanisms of Folding of Natural Elastic Materials. (Developments in Geotectonics, 11.) Pp.xx+406. ISBN-0-444-41496-7. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 122; \$49.95.

KRAUS, E. B. (edited by). Modelling and Prediction of the Upper Layers of the Ocean. (Proceedings of a NATO Advanced Study Institute.) (Pergamon Marine Series, Vol. 1.) ISBN-0-08-020610-7. (Oxford and New York: Pergamon Press, 1977.) Hard cover £10.95; Flexi cover £6.95.

MANGHNANI, Muri H., and AKIMOTO, Syun-Iti (edited by). High-Pressure Research: Applications in Geophysics. Pp.xv+642. ISBN-0-12-468750-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £29.50; £20.95.

McCALL, G. J. H. (edited by). The Archean: Search for the Beginning. Pp.x+505. ISBN-0-87933-128-3. (Stroudsburg, Penn.: Dowden, Hutchinson and Ross, Inc., 1977. Distributed by Halstead Press, a Division of John Wiley and Sons, Inc., New York and Chichester.) £40.

MONIN, Andrey S., KAMENKOVICH, Vladimir, and KORT, Vladimir, G. Variability of the Oceans. English translation edited by John J. Lumley. Pp.xiii+241. ISBN-0-471-61328-2. (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) £14.95; £25.35.

MUELLER, Robert F., and SAXENA, Surendra K. Chemical Petrology, with Applications to The Terrestrial Planets and Meteorites. Pp.xii+394. ISBN-3-540-90196-5. (New York and Berlin: Springer-Verlag, 1977.) DM 72.80; \$32.10.

NIHOUL, Jacques C. J. (edited by). Bottom Turbulence. (Proceedings of the 8th International Liège Colloquium on Ocean Hydrodynamics.) (Elsevier Oceanography Series, 19.) Pp.xiv+306. ISBN-0-444-41574-2. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 98; \$39.95.

ROCKER, Norman Hurd. Transient Waves in Visco-Elastic Media. (Developments in Solid Earth Geophysics, 10.) Pp.x+278. ISBN-0-444-41526-2. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 120; \$48.95.

SHEPARD, Francis P. Geological Oceanography: Evolution of Coasts, Continental Margins, and the Deep-Sea Floor. Pp.ii+214+8 plates. ISBN-0-8448-1064-9. (New York: Crane, Russak and Company, Inc., 1977.) \$10.50.

SMITH, A. G., and BRIDEN, J. C. Mesozoic and Cenozoic Paleogeographical Maps. (Cambridge Earth Science Series.) Pp.63. ISBN-0-521-29117-8. (Cambridge, London and New York: Cambridge University Press, 1977.) £1.95.

SUFFET, I. H. (edited by). Fate of Pollutants in the Air and Water Environments. Part 1: Mechanism of Interaction Between Environments and Mathematical Modeling and The Physical Fate of Pollutants. Pp.xx+484. ISBN-0-471-83539-0. (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) £18.70; \$31.70.

SWAIN, F. M. (edited by). Stratigraphic Micropaleontology of Atlantic Basin and Borderlands. (Developments in Palaeontology and Stratigraphy, 6.) Pp.vii+603. ISBN-0-444-41554-8. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 122; \$49.95.

WORTHINGTON, L. V. On the North Atlantic Circulation. (The Johns Hopkins Oceanographic Studies, No. 6.) Pp.109. ISBN-0-8018-1742-0. (Baltimore and London: The Johns Hopkins University Press, 1976.) £12.40.

YALIN, M. Selim. Mechanics of Sediment Transport. Second edition. Pp.xiv+298. ISBN-0-08-021162-3. (Oxford and New York: Pergamon Press, 1977.) \$35.

Biological Sciences

ABRAHAMSSON, S., and PASCHER, I. (edited by). Structure of Biological Membranes. Pp.xi+580. ISBN-0-306-33704-5. (New York and London: Plenum Press, 1977.) \$59.40.

ALVIN, Kenneth L. The Observer's Book of Lichens. Pp.188 (53 illustrations in colour and 109 black and white photographs and diagrams). ISBN-0-7232-1566-9. (London: Frederick Warne (Publishers), Ltd., 1977.) £1.10 net.

ANDREWS, S. Mahala, MILES, R. S., and WALKER, A. D. (edited by). Problems in Vertebrate Evolution. (Linnean Society Symposium Series, No. 4. Essays presented to Professor T. S. Westoll, FRS, FLS.) Pp.xi+411. ISBN-0-12-059950-3. (London and New York: Academic Press 1977. Published for the Linnean Society of London.) £18.50; \$36.10.

BAER, Adela S. (edited by). Heredity and Society: Readings in Social Genetics. Second edition. Pp.xvii+460. ISBN-0-02-305160-4. (New York: Macmillan Publishing Co., Inc.; London: Collier Macmillan Publishers, 1977.) £6.

BARRÉTT, A. J. (edited by). Proteinases in Mammalian Cells and Tissues. (Research Monographs in Cell and Tissue Physiology, Vol. 2.) Pp.xx+735. ISBN-0-7204-0619-6. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) Dfl. 196; \$79.95.

BLACHE, J. Leptocéphales des Poissons Anguilliformes dans la Zone Sud du Golfe de Guinée. (Faune Tropicale, XX.) Pp.377. ISBN-2-7099-0459-4. (Paris: Office de la Recherche Scientifique et Technique Outre-Mer, 1977.) 115 francs.

BOGORAD, L., and WEIL, J. H. (edited by). Nucleic Acids and Protein Synthesis in Plants. (NATO Advanced Study Institutes Series. Series A: Life Sciences, Vol. 12.) Pp.xi+417. ISBN-0-306-35612-0. (New York and London: Plenum Press, 1977. Published in co-operation with NATO Scientific Affairs Division.) \$47.40.

BLOOM, Alan. Perennials in Island Beds: a Selection of the Best Hardy Plants Pp.94+16 plates. ISBN-0-571-10892-X. (London: Faber and Faber, Ltd., 1977.) £3.95.

BOLD, Harold C. The Plant Kingdom. Fourth edition (Prentice-Hall Foundations of Modern Biology Series.) Pp.x+310. ISBN-0-13-680389-X. (Englewood Cliffs NJ and Hemel Hempstead, Herts: Prentice-Hall, 1977.) \$10.10; £6.35.

BOURNE, G. H., and DANIELLI, J. F. (edited by). Assisted by JEON, K. W. International Review of Cytology, Vol. 49. Pp.ix+394. ISBN-0-12-364349-X. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$34.50; £24.50.

BREAST-FEEDING AND THE MOTHER. (Ciba Foundation Symposium 45, New Series.) Pp.280. ISBN-0-444-15241-5. (Amsterdam, Oxford and New York: Elsevier/Excerpta Medica/North-Holland, 1976.) Dfl. 54; \$20.95 clothbound. Dfl. 30; \$11.50 paperback.

BROOM, Donald. The Wonderful World of Birds and Their Behaviour. Pp.96. ISBN-0-600-31391-3. (London and New York: Hamlyn 1977.) £2.95.

BURNS, Robert D., and STILES, Karl A. Laboratory Explorations in General Zoology. Sixth edition. Pp.viii+390. ISBN-0-02-317160-X. (New York: Macmillan Publishing Co., Inc.; London: Collier Macmillan Ltd., 1977.) £5.25.

BURTON, Maurice, and BURTON, Robert. Inside the Animal World: An Encyclopedia of Animal Behaviour. Pp.316. ISBN-0-333-19213-3. (London and Basingstoke: Macmillan London Limited, 1977.) £6.95 net.

BURTON, Robert. The Seashore and Its Wildlife. Pp.128. ISBN-0-85613-239-X. (London: Orbis Publishing, Ltd., 1977.) £5.95.

BUTT, W. R. Hormone Chemistry. Vol. 2: Steroids, Thyroid Hormones, Biogenic Amines and Prostaglandins. Second revised edition. Pp.272. ISBN-8531-038-2. (Chichester: Ellis Horwood, Ltd., New York: Halsted Press, a Division of John Wiley and Sons, Inc., 1976. World Distributors: John Wiley and Sons.) £16; \$30.40.

CARR, J. D. The South African Acacias. Pp.323. (Johannesburg, London, Manzini: Conservation Press (Pty.), Ltd., 1976.) R.20.

CHARLES-DOMINIQUE, Pierre. Ecology and Behaviour of Nocturnal Primates. Translated by R. D. Martin. Pp.x+277. ISBN-0-7156-0983-1. (London: Gerald Duckworth and Co., Ltd., 1977.) £12.50 net.

COFFEY, David J. The Encyclopedia of Sea Mammals. American Consultant Editors: David K. Caldwell and Melba C. Caldwell. Pp.223. ISBN-0-246-10979-3. (London: Hart-Davis, MacGibbon, 1977.) £12.

COLE, H. H., and CUPPS, P. T. (edited by). Reproduction in Domestic Animals. Third edition. Pp.xvi+665. ISBN-0-12-179252-8. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$39.50; £28.05.

CROLL, Neil A., and MATTHEWS, Bernard E. Biology of Nematodes. (Tertiary Level Biology.) Pp.vii+201. ISBN-0-216-90294-0. (Glasgow and London: Blackie and Son, Ltd., 1977.) £6.25.

DAVITASHVILI, L. Sh. The Theory of Evolution, Vol. 1. Pp.477. In Russian. (Tbilisi: Metsniereba, 1977.) 6.20 rubles.

DOBZHANSKY, Theodosius, AYALA, Francisco J., STEBBINS, G. Ledyard, and VALENTINE, James W. Evolution. Pp.xiv+572. ISBN-0-7167-0572-9. (San Francisco and Reading: W. H. Freeman and Co., 1977.) £11.60.

ENGEL, Paul C. Enzyme Kinetics: The Steady-State Approach. (Outline Studies in Biology.) Pp.96. ISBN-0-470-99097-X. (London: Chapman and Hall, 1977. Distributed in the USA by Halsted Press, a Division of John Wiley and Sons, Inc., New York.) £1.7.

EPSTEIN, H. Domestic Animals of Nepal. Pp.xii+131+112 plates. ISBN-0-8419-0242-X. (New York: Holmes and Meier Publishers, Inc.; London: Noonan Hurst, Ltd., 1977.) £27.50; £17.

ESAU, Katherine. Anatomy of Seed Plants. Second edition. Pp.xx+550. ISBN-0-471-24520-8. (New York and London: John Wiley and Co., 1977.) £11.50; £21.25.

FISHER, K. D., and NIXON, A. U. (edited by). The Science of Life: Contributions of Biology to Human Welfare. Pp.xxiv+358. ISBN-0-306-20025-2. A Plenum/Rosetta edition. (New York: Plenum Publishing Corporation, 1977.) \$9.

FRAENKEL-CONRAT, Heinz, and WAGNER, Robert R. (edited by). Comprehensive Virology, Vol. 7: Reproduction—Bacterial DNA Viruses. Pp.xii+300. ISBN-0-306-35147-1. (New York and London: Plenum Press, 1977.) \$30.

FRAENKEL-CONRAT, Heinz, and WAGNER, Robert R. (edited by). Comprehensive Virology, Vol. 8: Regulation and Genetics—Bacterial DNA Viruses. Pp.xii+350. ISBN-0-306-35148-X. (New York and London: Plenum Press, 1977.) \$35.40.

FRIGERIO, Alberto, and GHISALBERTI, Emilio L. (edited by). Mass Spectrometry in Drug Metabolism. Pp.xiii+532. ISBN-0-306-31018-X. (New York and London: Plenum Press, 1977.) \$51.

FRINGS, Hubert, and FRINGS, Mable. Animal Communication. Second edition, revised and enlarged. Pp.xi+207. ISBN-0-8061-1392-8. (Norman: University of Oklahoma Press, 1977.) Cloth \$9.95; Paper \$4.95.

GILPIN, Michael E. Group Selection in Predator-Prey Communities. Pp.xiii+109. (Monographs in Population Biology.) (Princeton, NJ: Princeton University Press, 1975.) Cloth £7.90; Paper £3.80.

- GOLUB, Edward S. *The Cellular Basis of the Immune Response: An Approach to Immunobiology*. Pp.x+278. ISBN-0-87893-210-0. (Sunderland, Mass.: Sinauer Associates, Inc.; Reading: W. H. Freeman and Co., Ltd., 1977.) £6.50.
- GORDON, Malcolm S. In collaboration with BARTHOLOMEW, George A., GRINNELL, Alan D., JORGENSEN, C. Barker, and WHITE, Fred N. *Animal Physiology: Principles and Adaptations*. Third edition. Pp.xx+699. ISBN-0-02345330-3. (New York: Macmillan Publishing Co., Inc.; London: Collier Macmillan Publishers, 1977.) £12.
- GRANT, Verne. *Organismic Evolution*. Pp.xii+418. ISBN-0-7167-0372-6. (San Francisco and Reading: W. H. Freeman and Company, 1977.) £11.70.
- GUIGNARD, J. L. *Abrégé de Botanique: à l'Usage des Etudiants en Pharmacie*. Troisième édition, Révisée et augmentée. Pp.xii+257. ISBN-2-22546-410-3. (Paris and New York: Masson et Cie., 1977.) 49 francs.
- HART, Gavin. *Human Sexual Behavior*. (Carolina Biology Readers, No. 94. Pp.31. ISBN-0-89278-294-3. (Burlington, North Carolina: Scientific Publications Division, Carolina Biological Supply Company, 1977.) n.p.
- HARTL, Daniel L. *Our Uncertain Heritage: Genetics and Human Diversity*. Pp.xvi+494. ISBN-0-397-47366-4. (Philadelphia and New York: J. B. Lippincott Company, 1977. Distributed in the UK by Blackwell Scientific Publications.) £11.20.
- HATHWAY, D. E. (senior reporter). *Foreign Compound Metabolism in Mammals*. Vol. 4. (A Review of the Literature Published during 1974 and 1975. A Specialist Periodical Report.) Pp.xiii+411. ISBN-0-85186-038-9. (London: The Chemical Society, 1977. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts.; American Chemical Society, 1155 Sixteenth Street, NW, Washington, DC.) £27.50; \$55.
- HANDBOOK ON THE PREVENTION AND TREATMENT OF SCHISTOSOMIASIS. (A translation of a Chinese publication.) (A Publication of the Geographic Health Studies, John E. Fogarty International Center for Advanced Study in the Health Sciences.) DHEW Publication No. (NIH) 77-1290. Pp.xii+172. (Washington, DC: US Government Printing Office, 1977.)
- HEYMER, Armin. *Ethnologisches Wörterbuch/Ethological Dictionary/Vocabulaire Ethnologique*. Pp.237. ISBN-3-489-66336-5. (Berlin und Hamburg: Verlag Paul Parey, 1977.) DM28.
- HUNSAKER, II, Don (edited by). *The Biology of Marsupials*. Pp.xv+537. ISBN-0-12-362250-6. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$36; £25.55.
- HUXLEY, Anthony (botanical editor). *The Encyclopedia of the Plant Kingdom*. Pp.240. ISBN-0-600-33134-2. (London and New York: Hamlyn, 1977.) £4.95.
- JAMIESON, G. A., and ROBINSON, D. M. (edited by). *Mammalian Cell Membranes*. Vol. 4: Membranes and Cellular Functions. Pp.262. ISBN-0-408-70774-7. Vol. 5: Responses of Plasma Membranes. Pp.316. ISBN-0-408-70775-5. (London and Boston, Mass.: Butterworths, 1977.) £18 each volume.
- JOHANSSON, S. G. O., STRANDBERG, Kjell, and UVNAS, Borge (edited by). *Molecular and Biological Aspects of the Acute Allergic Reaction*. (Nobel Foundation Symposium.) Pp.xi+463. ISBN-0-306-33703-7. (New York and London: Plenum Press, 1976.) \$47.40.
- LINDSAY, Robert D. (edited by). *Computer Analysis of Neuronal Structures*. (Computers in Biology and Medicine.) Pp.xvi+219. ISBN-0-306-30964-5. (New York and London: Plenum Press, 1977.) £27.
- MACLEAN, Norman. *The Differentiation of Cells*. (Genetics—Principles and Perspectives: a Series of Texts.) Pp.viii+216. ISBN-0-7131-2567-5. (London: Edward Arnold (Publishers), Ltd., 1977.) Boards £12; Paper £5.95.
- MANSON, Lionel A. (edited by). *Biomembranes*. Vol. 8. Pp.xii+244. ISBN-0-306-39808-7. (New York and London: Plenum Press, 1976.) £27.
- MOLLER, Göran (edited by). *Idiotypes on T and B Cells*. (Immunological Reviews. Vol. 34.) Pp.164. ISBN-87-16-02487-7. (Copenhagen: Munksgaard, 1977.) D.kr.87.
- MOSBACH, Klaus (edited by). *Methods in Enzymology*. Vol. 44: Immobilized Enzymes. Pp.xx+999. ISBN-0-12-181944-2. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$48; £34.10.
- MOYLE, John B., and MOYLE, Evelyn W. *Northland Wild Flowers: a Guide for the Minnesota Region*. Pp.vii+236. ISBN-0-8166-0806-7. (Minneapolis: University of Minnesota Press, 1977.) \$12.95.
- NORTH, Wheeler J. *Underwater California*. With sections on underwater photography by Robert Hollis. (California Natural History Guides: 39.) Pp.276+8 plates. (Berkeley, Los Angeles and London: University of California Press, 1976.) Cloth £8; Paper £4.75.
- OWEN, Myrlyn. *Wildfowl of Europe*. Foreword by Sir Peter Scott. Colour plates by Hilary Burn. Pp.256. ISBN-0-333-18701-6. (London and Basingstoke: Macmillan London, Ltd., 1977.) £15 net.
- PARKER, H. W. Sankes: a Natural History. Second edition. Revised and enlarged by A. G. C. Grandison. Pp.108+16 plates. (London: British Museum (Natural History); Ithaca: Cornell University Press, 1977.) £4.95.
- PERRY, S. V., MARGRETH, A., and ADELSTEIN, R. S. (edited by). *Contractile Systems in Non-Muscle Tissues*. (Proceedings of the International Symposium, Bressanone, Italy, 19–22 September 1976.) Pp.xi+360. ISBN-0-7204-0616-1. (Amsterdam, Oxford and New York: North-Holland Publishing Company, 1976.) Dfl. 80; \$32.75.
- PRIME, C. T. *Plant Life*. (Collins Countryside Series.) Pp.160+24 plates. ISBN-0-00-219111-3. (London and Glasgow: William Collins, Sons and Co., Ltd., 1977.) £2.50.
- PURSEY, Helen L. *The Wonderful World of Mushrooms and Other Fungi*. 96. ISBN-0-600-36248-5. (London and New York: Hamlyn, 1977.) £2.95.
- ROBERT, L., and ROBERT, A. M. (edited by). *Adipose Tissue: Lipids and the Intercellular Matrix*. (Frontiers of Matrix Biology. Vol. 2.) Pp.x+156. ISBN-3-8055-2223-1. (Basel, London and New York: S. Karger, 1976.) Sfr./DM 91; \$35.
- ROMANS, Robert C. (edited by). *Geobotany*. Pp.viii+308. ISBN-0-306-31007-4. (New York and London: Plenum Press, 1977.) \$35.40.
- ROSEN-RUNGE, Edward C. *The Processes of Spermatogenesis in Animals*. (Developmental and Cell Biology. Vol. 5.) Pp.viii+214. ISBN-0-521-21233-2. (Cambridge, London and New York: Cambridge University Press, 1977.) £15.50.
- SCHAUENSTEIN, E., ESTERBAUER, H., ZOLLNER, H. Aldehydes in Biological Systems: Their Natural Occurrence and Biological Activities. Translated by P. H. Gore. Pp.205. ISBN-0-85086-059-8. (London: Pion, Ltd., 1977.) £9.
- TWEEDIE, Michael. *Insect Life*. (Collins Countryside Series.) Pp.192+24 plates. ISBN-0-00-219343-4. (London and Glasgow: William Collins, Sons and Co., Ltd., 1977.) £2.50.
- WEIGLE, William O. (edited by). *Contemporary Topics in Immunobiology*. Vol. 5. Pp.xvi+341. ISBN-0-306-37805-1. (New York and London: Plenum Press 1976.) \$35.40.
- WYNN, Ralph M. (edited by). *Biology of the Uterus*. Pp.xix+748. ISBN-0-306-30949-1. (New York and London: Plenum Press, 1977.) \$56.10.
- BARKER, William H. (edited by). *Preventive and Community Medicine in Primary Care*. (A Conference Sponsored by The John E. Fogarty International Center for Advanced Study in the Health Sciences, and the Association of the Teachers of Preventive Medicine.) Pp.xxii+125. (Bethesda, Md.: US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, 1976. For sale by US Government Printing Office, Washington, DC.) \$4.90.
- BECKER, E. Lovell (edited by). *Seminars in Nephrology*. Pp.xii+194. ISBN-0-471-01804-X. (New York and London: John Wiley and Sons, 1977.) \$16.70; £9.70.
- BERCI, George (edited by). *Endoscopy*. Pp.xxi+805. ISBN-0-8385-2216-5. (New York: Appleton-Century-Crofts; Hemel Hempstead: Prentice-Hall International, 1976.) \$99.70; £62.80.
- BLUM, Kenneth (edited by). In Association with BARD, Diana L., and HEMILTON, Murray G. *Alcohol and Opiates: Neurochemical and Behavioral Mechanisms*. (Proceedings of a Symposium held in New York, March 26–28, 1976.) Pp.xix+403. ISBN-0-12-108450-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$17.50; £12.40.
- BOUHUY, Arend. *The Physiology of Breathing: a Textbook for Medical Students*. Pp.xvi+352. ISBN-0-8089-0984-3. (New York and London: Grune and Stratton, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$12.50; £8.85.
- BULLA, Jr., Lee A., and CHENG, Thomas C. (edited by). *Comparative Pathobiology*. Vol. 1: Biology of the Microsporidia. Pp.xvi+371. ISBN-0-306-38121-4. (New York and London: Plenum Press, 1976.) \$45.
- BYRDE, R. J. W., and WILLETS, H. J. *The Brown Rot Fungi of Fruit: Their Biology and Control*. Pp.xv+171. ISBN-0-08-019740. (Oxford and New York: Pergamon Press, 1977.) \$12.
- CAEN, Jacques P., CRONBERG, Stig, and KUBISZ, Peter. *Platelets: Physiology and Pathology*. Pp.vii+136. ISBN-0-913258-46-6. (New York: Stratton Intercontinental Medical Book Corporation, 1977.) \$17.50.
- CARSTENSEN, Jens T. *Pharmaceutics of Solids and Solid Dosage Forms*. Pp.xii+256. ISBN-0-471-13726-X. (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) \$21.50; £12.40.
- CHARLEY, Vernon L. S. *Black Current Juice Processing Technology*. (Fruit Juices and other Fruit Products: a Series of Monographs.) Pp.132. (Braunschweig: Verlag Günter Hempel, 1977.)
- CHRISTIE, A. B., and CHRISTIE, Mary C. *Food Hygiene and Food Hazards for All Who Handle Food*. Pp.232. ISBN-0-571-10902-0. (London: Faber and Faber, Ltd., 1977.) Cloth £4.95; Paper £2.50.
- DAY, Peter R. (edited by). *The Genetic Basis of Epidemics in Agriculture*. (Annals of the New York Academy of Sciences, Vol. 287.) Pp.vii+400. ISBN-0-80972-033-9. (New York: New York Academy of Sciences, 1977.) n.p.
- DUPLAN, J. F. (edited by). *Radiation-Induced Leukemogenesis and Related Viruses*. (Proceedings of the International Symposium held in Bordeaux, 7–9, December 1976. INSERM Symposium No. 4.) Pp.xxi+331. ISBN-0-7204-0634-X. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1977.) Dfl. 73; \$29.95.
- EVANS, A. S. *Viral Infection in Humans*. ISBN-0-471-99435-9. (Chichester: John Wiley and Sons, Ltd., 1977. Market Restriction: UK, Europe, Middle East, Africa.) £23; \$44.
- THE FATE OF DRUGS IN THE ORGANISM; a Bibliographic Survey. Vol. 4. Compiled by Société Française des Sciences et Techniques Pharmaceutiques Working Group, under the chairmanship of J. Hirtz. Pp.xvii+612. ISBN-0-8247-6587-7. (New York and Basel: Marcel Dekker, Inc., 1977.) Sfrs. 240.
- FEACHEM, Richard, McGARRY, Michael, and MARA, Duncan. (edited by). *Water, Wastes and Health in Hot Climates*. Pp.xvi+309. ISBN-0-471-99410-3. (London and New York: Wiley-Interscience, John Wiley and Sons, 1977.) £13.40; \$22.
- FOLKERS, K., and Yamamura, Y. (edited by). *Biomedical and Clinical Aspects of Coenzyme Q*. (Proceedings of the International Symposium on Coenzyme Q, held at Lake Yamanaka, Japan, September 16/17, 1976, a Naito Foundation Symposium.) Pp.xi+315. ISBN-0-444-41576-9. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl.80; \$32.75.
- FRIGERIO, Alberto (edited by). *Advances in Mass Spectrometry in Biochemistry and Medicine*. Vol. 2. (Proceedings of the 3rd International Symposium, Mario Negri Institute for Pharmacological Research, June 1975, Milan, Italy.) Pp.609. ISBN-0-470-99039-2. (New York: SP Books Division of Spectrum Publications, Inc., 1977. Distributed by Halsted Press, a Division of John Wiley and Sons, New York and London.) \$0; £35.
- GAMAN, P. M., and SHERRINGTON, K. B. *The Science of Food: an Introduction to Food Science, Nutrition and Microbiology*. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xi+300. ISBN-0-08-019947-X. (Oxford and New York: Pergamon Press, 1977.) \$8.95.
- GREENWALT, Tibor J., and JAMIESON, G. A. (edited by). *The Granulocyte: Function and Clinical Utilization*. (Progress in Clinical and Biological Research, Vol. 13.) Pp.xiii+341. ISBN-0-8451-0013-0. (New York: Alan R. Liss, Inc., 1977.) \$30.
- GROTE, Jürgen, RENEAU, Daniel, and THEWS, Gerhard (edited by). *Oxygen Transport to Tissue-II*. (Advances in Experimental Medicine and Biology, Vol. 75.) Pp.xxiii+781. ISBN-0-306-39075-2. (New York and London: Plenum Press 1976.) \$98.40.
- HARDY, R. W. F. (general editor.) GIBSON, A. H. (section IV editor.) *A Treatise on Dinitrogen Fixation*. Section IV: Agronomy and Ecology. Pp.xii+527. ISBN-0-471-02343-4. (section 4.) (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) \$39.40; £23.
- HARNAD, Stevan, DOTY, Robert W., JAYNES, Julian, GOLDSTEIN, L., and KRAUTHAMER, George (edited by). *Lateralization in the Nervous System*. Pp.xlviii+537. ISBN-0-12-325750-6. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$21.50; £15.25.
- HYLTON, William H. (edited by). *The Rodale Herb Book: How to Use, Grow, and Buy Nature's Miracle Plants*. (An Organic Gardening and Farming Book.) Pp.653. ISBN-0-87857-076-4. (Emmaus, Pa., and Berkhamsted: Rodale Press Book Division, 1977.) £9.50 net.
- IOACHIM, Harry L. (edited by). *Pathobiology Annual*. Vol. 6. Pp.x+419. ISBN-0-8385-7737-7. (New York: Appleton-Century-Crofts; Hemel Hempstead: Prentice-Hall International, 1976.) \$36.20; £22.80.
- KOBAYASHI, M., HELLMAN, L. M., and CROMB, E. *Atlas of Ultrasonography in Obstetrics and Gynecology*. Pp.viii+443. ISBN-0-8385-0139-7. (New York: Appleton-Century-Crofts, a Publishing Division of Prentice-Hall, Inc.; Hemel Hempstead: Prentice-Hall International, 1972.) \$36.20; £22.80.
- LANDERS, III, Maurice B., DOWLING, John E., and LATIES, Alan. (edited by). *Retinitis Pigmentosa: Clinical Implications of Current Research*. (Advances in Experimental Medicine and Biology, Vol. 77.) Pp.xviii+266. ISBN-0-306-39077-9. (New York and London: Plenum Press, 1977.) \$30.
- LEAKEY, C. L. A., and WILLIS, J. B. (edited by). *Food Crops of the Lowland Tropics*. Pp.xiv+345. ISBN-0-19-854517-7. (Oxford, London and New York: Oxford University Press, 1977.) £9 net.
- LEWIS, Walter H., and ELVIN-LEWIS, M. P. F. *Medical Botany: Plants Affecting Man's Health*. Pp.xv+515. ISBN-0-471-53320-3. (New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) \$31.65; £18.
- MCOMAS, Alan J. *Neuromuscular Function and Disorders*. Pp.x+364. ISBN-0-407-00058-5. (London and Boston, Mass.: Butterworths, 1977.) £19.50.
- McMINN, R. M. H., and HUTCHINGS, R. T. *A Colour Atlas of Human Anatomy*. Pp.352. ISBN-0-7234-0709-6. (London: Wolfe Medical Publications, Ltd., 1977.) £15.
- MULLER, Mathias M., KAISER, Erich, and SEEGLMILLER, J. Edwin. (edited by). *Purine Metabolism in Man—I: Regulation of Pathways and Enzyme Defects*. (Advances in Experimental Medicine and Biology, Vol. 76A.) Pp.xxii+641. ISBN-0-306-39089-2 (Vol. 76A). (New York and London: Plenum Press, 1977.) \$59.40.

Applied Biological Science

- ALABASTER, John S. (edited by). *Biological Monitoring of Inland Fisheries*. (Proceedings of a Symposium of the European Inland Fisheries Advisory Commission, Helsinki, Finland, 7–8 June, 1976.) Pp.xvi+226. ISBN-0-85334-719-0. (London: Applied Science Publishers, Ltd., 1977. Published by arrangement with the Food and Agriculture Organization of the United Nations.) £15.
- ALLEN, Jr., Marshall B., and MAHESH, Virendra B. (edited by). *The Pituitary: a Current Review*. (Proceedings of a Symposium held at the Medical College of Georgia, Augusta, Georgia, May 20–22, 1976.) Pp.xv+545. ISBN-0-12-051850-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$25; £17.75.
- ARNOLD, Thomas M., DALRYMPLE, Dana G., and RUTTAN, Vernon W. (edited by). *Resource Allocation and Productivity in National and International Agricultural Research*. Pp.xviii+617. ISBN-0-8166-0805-9. (Minneapolis: University of Minnesota Press, 1977.) \$25.

- MULLER, Mathias M., KAISER, Erich, and SEEGLER, J. Edwin. (edited by). *Purine Metabolism in Man—II: Physiology, Pharmacology, and Clinical Aspects*. (Advances in Experimental Medicine and Biology, Vol. 76B.) ISBN-0-306-39090-6. (Vol. 76B.) (New York and London: Plenum Press, 1977.) \$42.
- PERKINS, W. J. (edited by). *Biomedical Computing*. Pp.ix + 362. ISBN-0-272-79401-5. (London: Pitman Medical Publishing Co., Ltd., 1977.) £20 net.
- PERLAM, D. (edited by). *Advances in Applied Microbiology*, Vol. 21. Pp.x + 300. ISBN-0-12-002621-X. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$23; £16.35.
- PISANO, John F., and AUSTEN, K. Frank (edited by). *Chemistry and Biology of the Kalikrein-Kinin System in Health and Disease*. (Conference held at the National Institutes of Health, Bethesda, Maryland, October 20–23, 1974. Fogarty International Center Proceedings No. 27) Pp.xxxviii + 612. (Washington, DC: US Government Printing Office, 1976.) \$12.
- RAJKA, E., and KOROSSY, S. (edited by). *Immunological Aspects of Allergy and Allergic Diseases*. Vol. 5: Clinical Aspects of Allergic Diseases. Pp.vii + 254. ISBN-0-306-37755-1. (London and New York: Plenum Press; Budapest: Akademiai Kiado, 1976.) \$42.
- RICHTER, G. W., and EPSTEIN, M. A. (edited by). *International Review of Experimental Pathology*, Vol. 17. Pp.vii + 250. ISBN-0-12-364917-X. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$25.50; £18.10.
- ROBERT, A. M., and ROBERT, L. (edited by). *Burkitt Lymphoma, Hemostasis and Interleukin Matrix*. (Barbara Robert Memorial.) (Frontiers of Matrix Biology, vol. 3.) Pp.xxiv + 270. ISBN-3-8055-2326-2. (Basel, London and New York: S. Karger, 1976.) SFr/DM 179; \$69.
- SANCHEZ, Pedro A. *Properties and Management of Soils in the Tropics*. Pp.x + 618. ISBN-0-471-75200-2. (New York and London: Wiley-Interscience, John Wiley and Sons, 1976.) \$38.10; £22.50.
- SMITH, Kenneth M. *Plant Viruses*. Sixth edition. Pp.viii + 241 + 24 plates. ISBN-0-470-98995-5. (London: Chapman and Hall; New York: Halsted Press, John Wiley and Sons, 1977.) £3.25.
- SOMMER, Sheldon C. (edited by). *Pathology Annual*, Vol. 11. Pp.x + 465. ISBN-0-8385-7745-8. (New York: Appleton-Century-Crofts; Hemel Hempstead: Prentice-Hall International, 1976.) \$32.35; £20.40.
- TRIGGLE, D. J., and TRIGGLE, C. R. *Chemical Pharmacology of the Synapse*. Pp.viii + 654. ISBN-0-12-700340-1. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) £20; \$43.75.
- UNDERWOOD, E. J. *Trace Elements in Human and Animal Nutrition*. Fourth edition. Pp.xii + 545. ISBN-0-72709065-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$29.50; £20.95.
- VOGEL, John H. K. (edited by). *Future Directions in the Management of Cardiac Disease: a Bicentennial Viewpoint*. (Advances in Cardiology, Vol. 20.) (7th Conference on Cardiovascular Disease in Snowmass-at-Aspen, Aspen, Colorado, January 11–13, 1976.) Pp.vi + 144. ISBN-3-8055-2412-9. (Basel, London and New York: S. Karger, 1977.) SFr/DM 75; \$29.
- WARREN, Kenneth S., and HOFFMAN, Donald B. *Schistosomiasis III: Abstracts of the Complete Literature 1963–1974*. Vol. 1: A through J. Pp.vi + 1–336. ISBN-0-470-15204-4(v.1). Vol. 2: K through Z. Pp.vi + 337–730. ISBN-0-470-98896-7. (v.2) (Washington, DC: Hemisphere Publishing Corporation, 1976. Distributed solely by Halsted Press, a Division of John Wiley and Sons, New York and London.) np.
- WEGELIUS, Otto, and PASTERNAK, Amos (edited by). *Amyloidosis*. (Proceedings of the Fifth Sigrid Juselius Foundation Symposium.) Pp.xvi + 605. ISBN-0-12-741350-2. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) £18; \$32.25.
- WEISS, David W. (edited by). *Immunological Parameters of Host-Tumor Relationships*, Vol. 4. Pp.221. ISBN-0-12-743554-5. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1976.) \$21.50; £15.25.

Psychology

- COHEN, David. *Psychologists on Psychology*. Pp.360. ISBN-0-7100-8502-8. (London and Henley: Routledge and Kegan Paul, 1977.) £6.95 net.
- JARVIK, Murray E. (edited by). *Psychopharmacology in the Practice of Medicine*. Pp.xvi + 553. ISBN-0-8385-7950-7. (New York and Hemel Hempstead: Appleton-Century-Crofts, 1977.) \$26.05; £16.40.
- KAZDIN, Alan E. *The Token Economy: a Review and Evaluation*. (The Plenum Behavior Therapy Series.) Pp.xvi + 342. ISBN-0-306-30962-9. (New York and London: Plenum Press, 1977.) £20.34.
- KREML, William P. *The Anti-Authoritarian Personality*. (International Series of Monographs in Experimental Psychology, Vol. 21.) Pp.xi + 118. ISBN-0-08-021063-5. (Oxford and New York: Pergamon Press, 1977.) £7.25; \$14.50.
- MACKENZIE, Brian D. *Behaviourism and the Limits of Scientific Method*. (International Library of Philosophy and Scientific Method.) Pp.xiv + 193. ISBN-0-7100-8543-5. (London and Henley: Routledge and Kegan Paul, Ltd., 1977.) £4.95.
- MOORE, Brian C. J. *Introduction to the Psychology of Hearing*. Pp.311. ISBN-0-333-19700-3. (London and Basingstoke: The Macmillan Press, Ltd., 1977.) Hard cover £10; Paper cover £4.95.
- SILVERMAN, Irwin. *The Human Subject in the Psychological Laboratory*. (Pergamon General Psychology Series.) Pp.xii + 151. ISBN-0-08-021079-1. (Oxford and New York: Pergamon Press, 1977.) np.
- SMITH, Donald E. P. *A Technology of Reading and Writing*. Vol. 3: The Adaptive Classroom. (Educational Psychology.) Pp.x + 320. ISBN-0-12-651703-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$14.50; £10.30.
- THOMPSON, Travis, and DEWS, Peter B. (edited by). *Advances in Behavioral Pharmacology*, Vol. 1. Pp.x + 267. ISBN-0-12-004701-2. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$16.50; £11.70.
- UZZIRIS, I. C., and WEIZMANN, F. (edited by). *The Structuring of Experience*. Pp.xiv + 449. ISBN-0-306-30961-0. (New York and London: Plenum Press, 1977.) \$30.
- VERNON, Philip E., ADAMSON, Georgina, and VERNON, Dorothy F. *The Psychology and Education of Gifted Children*. Pp.viii + 216. ISBN-0-416-84390-5. (London: Methuen and Co., Ltd., 1977.) Hardback £5.95; Paperback £2.95. (Pub. 14 Apr. 77).

Sociology

- BERK, Richard A., BRACKMAN, Harold, and LESSER, Selma. *A Measure of Justice: An Empirical Study of Changes in the California Penal Code, 1955–1971*. (Quantitative Studies in Social Relations.) Pp.xix + 312. ISBN-0-12-091550-2. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$17.50; £12.40.
- LEE, Ronald Demos (edited by). *In collaboration with EASTERLIN, Richard A., LINDERT, Peter H., and van de WALLE, Etienne. Population Patterns in the Past*. (Studies in Social Discontinuity.) Pp.x + 376. ISBN-0-12-441850-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$ 9; £13.50.
- LEINHARDT, Samuel (edited by). *Social Networks: a Developing Paradigm*. Pp.xxxiv + 465. ISBN-0-12-442450-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$17.50; £12.40.
- MENDELSON, Everett, WEINGART, Peter, and WHITLEY, Richard (edited by). *The Social Production of Scientific Knowledge*. (Sociology of the Sciences: a Yearbook, Vol. 1.) Pp.vi + 294. ISBN-0-90-277-077-8. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl.65; \$26.
- PHILLIPS, Derek L. *Wittgenstein and Scientific Knowledge: a Sociological Perspective*. Pp.xiv + 248. ISBN-0-333-21314-9. (London and Basingstoke: The Macmillan Press, Ltd., 1977.) £10 net.

History of Science

- BARRETT, Paul H. (edited by). *The Collected Papers of Charles Darwin*, Vol. 2. Pp.viii + 326. ISBN-0-226-13657-4. (Chicago and London: The University of Chicago Press, 1977.) n.p.
- BILINSKI, Bronislaw. *Il Pitagorismo di Niccolò Copernico*. (Accademia Polacca delle Scienze: Biblioteca e Centro di Studi a Roma. Conferenze, Fasc. 69.) Pp.184. (Wrocław, Warszawa, Krakow, Gdansk: Zakład Narodowy Imienia Ossolińskich Wydawnictwo Polskiej Akademii Nauk, 1977.) Cena: 65.-zł.
- BRITTAIN, James E. (edited by). *Turning Points in American Electrical History*. Pp.xi + 399. ISBN-0-471-02568-2. (New York: The Institute of Electrical and Electronics Engineers, Inc., 1977. Distributed by John Wiley and Sons, New York and London.) \$25.95.
- KRAJEWSKI, Wladyslaw. *Correspondence Principle and Growth of Science*. (Episteme, Volume 4.) Pp.xiv + 136. ISBN-90-277-0770-7. (Dordrecht and Boston, Mass.: D. Reidel Publishing Company, 1977.) Dfl.55; \$19.50.
- LESKY, Erna. *The Vienna Medical School of the 19th Century*. Pp.xv + 604 + 100 photographs. ISBN-0-8018-1908-3. (Baltimore and London: The Johns Hopkins University Press, 1976.) £16; \$26.40.
- ORD-HUME, Arthur W. J. G. *Perpetual Motion: The History of an Obsession*. Pp.235. ISBN-0-04-621024-5. (London: George Allen and Unwin, Ltd., 1977.) £5.50 net.
- SIVIN, Nathan (edited by). *Science and Technology in East Asia*. (History of Science Selection from ISIS.) Pp.xxiv + 260. ISBN-0-88202-161-3. (New York: Science History Publications, a Division of Neale Watson Academic Publications, Inc., 1977.) Cloth \$9.95; Paper \$5.95.

Anthropology

- GOOCH, Stand. *The Neanderthal Question*. Pp.xv + 178. ISBN-0-7045-0260-7. (London: Wildwood House, 1977.) £5.95 net.
- GOODMAN, Morris, TASHIAN, Richard E., and TASHIAN, Jeanne H. (edited by). *Molecular Anthropology: Genes and Proteins in the Evolutionary Ascent of the Primates*. (Advances in Primatology.) Pp.xiii + 466. ISBN-0-306-30948-3. (New York and London: Plenum Press, 1976.) \$42.
- GREGERSEN, Edgar A. *Language in Africa: An Introductory Survey*. (Library of Anthropology.) Pp.xvii + 237. ISBN-0-677-04380-5. (New York, Paris and London: Gordon and Breach, 1977.) £13.
- HOLE, Frank. *Studies in the Archaeological History of the Deh Luran Plain: The Excavation of Chagha Sefid*. Contributions by M. J. Kirkby and Colin Renfrew. (Memoirs of the Museum of Anthropology, University of Michigan, No. 9.) Pp.xiv + 369 (55 plates). (Ann Arbor: Museum of Anthropology, University of Michigan, 1977.) \$10.
- KUPER, Adam (edited by). *The Social Anthropology of Radcliffe-Brown*. Pp.viii + 296. ISBN-0-7100-8556-7. (London, Henley and Boston, Mass.: Routledge and Kegan Paul, Ltd., 1977.) Cloth £6.95; Paper £3.50.
- LANDY, David (edited by). *Culture, Disease, and Healing: Studies in Medical Anthropology*. Pp.xv + 559. ISBN-0-02-367390-7. (New York: Macmillan Publishing Co., Inc.; London: Collier Macmillan Publishers, 1977.) £10.50.
- SEBEOK, Thomas A. (edited by). *Native Languages of the Americas*, Vol. 2. Pp.xiv + 535. ISBN-0-306-37158-8 (v.2). (New York and London: Plenum Press, 1977.) \$51.
- STEWART, Frank Henderson. *Fundamentals of Age-Group Systems*. (Studies in Anthropology.) Pp.xiii + 381. ISBN-0-12-670150-4. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$26; £18.45.

Education

- BOLEY, Bruno A. (edited by). *Crossfire in Professional Education: Students, the Professors and Society* (Proceedings of a Conference, October 16–17, 1975, Evanston, Illinois, USA.) Pp.x + 108. ISBN-0-08-021429-0. (Oxford and New York: Pergamon Press, 1977.) £5.
- GOOD, Ronald G. *How Children Learn Science: Conceptual Development and Implications for Teaching*. Pp.xiii + 337. ISBN-0-02-344640-4. (New York: Macmillan Publishing Co., Inc.; London: Collier Macmillan Publishers, 1977.) £8.25.
- HEYWOOD, John, and MONTAGU-POLLOCK, Hubert. *Science for Arts Students: a Case Study in Curriculum Development*. (Research into Higher Education Monographs.) Pp.v + 123. ISBN-900868473. (Guildford, Surrey: Society for Research into Higher Education, Ltd., 1977.) £3.90.
- HUGHES-EVANS, David (edited by). *Environmental Education: Key Issues of the Future*. (Proceedings of the Conference held at the College of Technology, Farnborough, England.) Pp.x + 82. ISBN-0-08-021490-8. (Oxford and New York: Pergamon Press, 1977.) £3.95.

General

- ACKERMAN, Bruce A. *Private Property and the Constitution*. Pp.ix + 303. ISBN-0-300-02065-1. (New Haven and London: Yale University Press, 1977.) £9.40.
- BLAKEMORE, Colin. *Mechanics of the Mind*. (BBC Reith Lectures 1976.) Pp.viii + 208. ISBN-0-521-21559-5. (Cambridge, London and New York: Cambridge University Press, 1977.) Hardcover £10.50; Paperback £3.95.
- BRIGGS, Julia. *Night Visitors: The Rise and Fall of the English Ghost Story*. Pp.238. ISBN-0-571-11113-0. (London: Faber and Faber, Ltd., 1977.) £6.95 net.
- BROWN, Harold I. *Perception, Theory and Commitment: The New Philosophy of Science*. Pp.203. ISBN-0-913750-13-1. (Chicago: Precedent Publishing, Inc., 1977.) \$15.95.
- BURNS, Leland S., and GREBLER, Leo. *The Housing of Nations: Analysis and Policy in a Comparative Framework*. Pp.xv + 255. ISBN-0-333-19810-7. (London and Basingstoke: The Macmillan Press, Ltd., 1977.) £10.
- CHAPMAN, Antony J., and FOOT, Hugh C. (edited by). *It's a Funny Thing, Humour*. Pp.xv + 507. ISBN-0-08-021376-6. (Oxford and New York: Pergamon Press, 1977.) £12.50.
- CHARMINE, Susan E. *The Complete Raw Juice Therapy*. Pp.128. ISBN-0-722503-61-X. (Wellingborough, Northants: Thorsons Publishers, Ltd., 1977.) £1.95.
- CLARK, Stephen R. L. *The Moral Status of Animals*. Pp.221. ISBN-0-19-824578-5. (Oxford: Clarendon Press; London: Oxford University Press, 1977.) £5.95 net.
- CLAUS, George, and BOLANDER, Karen. *Ecological Sanity*. Pp.xv + 592. ISBN-0-679-50388-9. (New York: David McKay Company, Inc., 1977.) \$16.95.
- CORLISS, William R. (compiled by). *Handbook of Unusual Natural Phenomena*. Pp.542. ISBN-0-915554-01-1. (Glen Arm, Md.: The Sourcebook Project, 1977.) \$14.95.
- DESMOND, Ray. *Dictionary of British and Irish Botanists and Horticulturists, including Plant Collectors and Botanical Artists*. Historical Introduction by William T. Stearn. Pp.xv + 747. ISBN-0-85066-089-0. (London: Taylor and Francis, Ltd., 1977.) £40 net.
- Du BRIN, Andrew. *Casebook of Organizational Behavior*. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xi + 326. ISBN-0-08-020502-X. (Oxford and New York: Pergamon Press, 1977.) Hardcover £7; Flexicover £4.
- EILON, Samuel. *Aspects of Management*. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.viii + 162. ISBN-0-08-020969-6. (Oxford and New York: Pergamon Press, 1977.) £5.

obituary

W. Grey Walter

DR W. GREY WALTER, who had an international reputation for his work on brain function, died suddenly on 6 May 1977, aged 67.

William Grey Walter—Grey to his many friends—was born in Kansas City in 1910 of an American mother and an English father, Karl Walter, editor of the *Kansas City Star*. The family came to London in 1917 and Grey went to Westminster School and then to Cambridge where he obtained a first in natural sciences in 1931, and did postgraduate work on nerve physiology with Professor (now Sir Brian) Matthews.

In 1935 he joined Professor F. L. Golla at the Maudsley Hospital and started the work on brain function that was to occupy all of his working life. In 1936 almost by chance an opportunity arose to record the electrical activity of the brain (electroencephalogram, EEG) of a patient with a cerebral tumour, and the characteristic low frequency (1 Hz) activity was discovered. This work opened up a new era in the detection and localisation of brain lesions, using the EEG. During this same period Grey was recording the EEGs of many epileptic patients and showed that many of them were abnormal *between* fits.

In 1939 Golla moved to Bristol to open a research laboratory and clinic called the Burden Neurological Institute and took Grey Walter with him. Here Grey was able to expand his work and was responsible for many new developments and discoveries. He made the first instrument in Britain for electro-shock therapy (1939) and was co-worker in its early use; he opened up the first clinical EEG department in Britain (1940); he developed the first portable electroencephalograph and the first low frequency automatic wave analyser (1942); and he developed electrode probes for recording deep within the brain during neurosurgical operations (1941).

In 1943 he discovered the theta rhythm; and later used the stroboscope to activate brain abnormalities in epileptic patients, triggering the flash from the brain activity—a particularly effective form of bio-feedback. It was about this time that he investigated the relationship between the EEG and mental imagery; in 1947 he published a paper on the effects of sensory stimuli

on the EEG. In the same year he was awarded the ScD from Cambridge University for his outstanding scientific work. Although a physiologist, his work was often closely related to clinical practice and in 1949 to mark the importance of this aspect of his work he was awarded an honorary MD and made honorary Professor of the University of Aix-Marseille.

At the turn of the decade Grey Walter was attracted to the new science of cybernetics which gave opportunity of rich cross-fertilisation of many disciplines. He developed several fascinating models of behaviour of which the mechanical tortoise *Machina speculatrix* was the most famous. In his book *The Living Brain* (1953) he drew together many aspects of brain science. Its value was not so much scientific (as most of the work had been published elsewhere) but as one of the first successful attempts to present such concepts in a readable form to the non-scientist.

From the beginning Grey Walter was fascinated by the concepts of Pavlovian conditioning and its application to learning processes. Many and varied were the experiments on this theme throughout the years using the EEG as an indicator of brain function in behaviour, but it was not until the technological development of averaging devices in the early '60s, enabling brain responses to sensory stimuli to be extracted easily from the background EEG, that the crucial discovery was made. This was the observation that changes of cortical potentials occur as a person having been prewarned by one stimulus makes a response to a second. This Grey Walter called the contingent negative variation (CNV) and published the work in *Nature* in 1964. Grey discovered the CNV not with the open mind said to be used by scientists but by a stubborn prejudice and persistent, perceptive searching for what "had to be there."

By a cruel and ironic stroke of misfortune, Grey Walter was involved in a road traffic accident in 1970 and suffered severe brain injury. He was unconscious for about three weeks but gradually made a remarkable recovery. He was appointed Emeritus Consultant when he retired from the Burden Neurological Institute in 1975. In the same year the EEG Society, of which he was a founder member, struck a special Grey Walter Medal and pre-

sented the first award to Grey Walter himself for his outstanding achievements. In 1974 he was awarded the Oliver-Sharpey prize of the Royal College of Physicians.

In these days of high technology it is difficult to realise the primitive nature of the scientific tools available in the 1930s. The electroencephalogram as recorded from scalp electrodes is about 50 microvolts peak to peak and has a frequency range from DC to about 100Hz. Even today much care is needed in order to record the EEG in a hospital environment where electrostatic and electromagnetic interference causes many problems. When Grey Walter started, the balanced (push-pull) amplifier had just been developed by Tonnes and the electronic components needed for the amplification of the low frequency brain activity were somewhat bulky when available. All the early equipment was made by Grey himself and had to be treated with loving care. Much of his early work was done using a smoked drum as recorder and many disagreeable hours must have been spent coating the paper; not until 1939 did he obtain a three channel inkwriter that gave long uninterrupted records. Much of this technological development occurred during the war years when shortages of equipment were very acute.

The main significance of his early work was not technological, though this was impressive enough, but the introduction into clinical medicine in this country of a unique method of studying the *functioning* brain. Even with the introduction of the EMI scanner and computer techniques thirty years later the EEG remains the only non-invasive method of observing the brain in action on a second to second time scale. Perhaps not surprisingly, the application of these techniques to patients by a physiologist was not always welcomed by the medical profession and even today there are only one or two scientists without medical qualifications reading clinical EEGs.

But what of the man? Grey was generous and helpful to others, especially young scientists, but sometimes his behaviour seemed arrogant, especially with senior colleagues, in the way of many scientists when they know (or think) that they are right. The publication of the CNV was delayed for about a year while he made sure that it was not an artefact due to eye move-

ments. Even after the publication, many groups throughout the world doubted whether it was a cortical event; Grey's attitude was firm, 'Let them spend time doubting, it will take them longer to catch us up.' He was unconcerned about money and in the 1950s was content with such a low salary that the income tax inspector did not believe his declared income. Yet over the years Grey obtained hundreds of thousands of pounds for the equipment and work at the Burden Institute. I think that he considered that to be able to work on brain research was a privilege that had to be paid for and I am sure that he would have continued working for nothing if the necessity had arisen. Work was a major passion in his life and he would spend many, many hours in the laboratory trying out new ideas often without any real lead to follow, persisting in the belief that discoveries are made in the laboratory and not at the desk or committee meetings. It is a pity that there are so few like him.

R. Cooper

Frank Green

DR FRANK GREEN, CBE, MD, who died on 30 April 1977 in his 77th year, had lived his professional life as a research administrator. From 1929 to 1955 he served the Medical Research Council rising through the various grades from Medical Officer to Principal and at the end of his period was Publications Editor. Their special reports were perhaps appropriately named "Green" books.

He worked under a succession of Secretaries from Sir Walter Fletcher and Sir Edward Mellanby to Sir Harold Himsworth. In the pre-war period of his MRC service the annual budget, allocated directly by the Privy Council, rose from approximately £150,000 to nearly £200,000. The organisation remained small, intimate and friendly and its relationships with a small band of research workers were personal, smooth running and inexpensive. Frank got to know them all and his wife Joyce acted as a charming hostess to many of us.

The MRC then occupied a pleasant old Georgian house at 38 Old Queen Street and the Council acted with an ease of judgement and the lightest of control. Research was still cheap, equipment often home made at laboratory benches, and grantees were trusted to spend their modest (often three-figure) allocations to the advantage of their experiments, which were, in those days, usually pursued with their own hands, with very limited technical help. When the War ended in 1945 the Treasury began to intervene in the allocations which in 1946 reached half

a million pounds, and were subsequently multiplied to two million by 1955. The MRC was now growing into a larger and less informal body, personal contacts became less easy to maintain, and the channels of communication became more complex.

Frank then side-stepped into the growing Wellcome Trust thus coming back into a much smaller organisation, in which he was in continuous daily contact with the chairman, Sir Henry Dale, the doyen of medical science of our era. The Wellcome funds at that time were limited to a few hundred thousand pounds and decisions of great responsibility fell largely to Dale's admirable scientific judgement combined with Green's personal knowledge of research applicants.

The allocations in those days were mainly to provide for costly equipment such as electron microscopes and many new laboratories of which the Medical Schools were in dire need. Indeed with out help from the Wellcome Trust medical research in our Universities in the immediate post-war years would have been severely hampered. Frank as the Executive Secretary was thus a trusted and key figure approached by everybody in need of supplements. Extrapolating backwards through his experience of the MRC he had indeed been a key figure in facilitating almost the whole development of British medical research from 1930 into the present era. His experience as Editor in the MRC gave him a capacity to work hard on drafts and produce his reports in impeccable English.

He was frequently invalided, often crippled by bronchitis and asthma. He worked to the limit of his capacity until recurrent illnesses each winter made it difficult for him to continue with his growing responsibilities while other physical disabilities also made him less mobile. When he relinquished his advisory services to the Wellcome Trust he gave part time services to the Science Committee of the growing British Heart Foundation. Once again his knowledge of personalities and his experience in judging applications helped to create a system in which the expertise of the various committees was always supplemented by consultation with appropriate experts in collateral fields. He thus epitomised in himself the ethics of research development and support based on the highest judgement of achievement and an intimate knowledge not only of the applicants and their sponsors but also of suitable referees.

He endured a long physical illness with patience supported and nursed by his loyal and devoted wife who always welcomed visits from their old friends.

Sir John McMichael

M. F. Lucas Keene

PROFESSOR MARY FRANCES LUCAS KEENE, doyenne of British anatomists, was born 15 August 1885 and died 9 May 1977. Her professional life was dedicated to the Royal Free Hospital School of Medicine (formerly London School of Medicine for Women), to the interests of the medical profession and the enhancement of the status of women therein. A brilliant undergraduate in the School she attracted encouragement from Stanley Boyd and F. G. Parsons and after adopting a career in anatomy was later influenced by F. Wood Jones. She graduated MB, BS in 1911 and joined the School's anatomy department to serve it with distinction as Lecturer in Embryology and Senior Demonstrator of Anatomy (1914), Lecturer and Head of Department (1919) and University Professor (1924-51). On retirement she was elected Emeritus Professor in the University of London.

Her principal researches were neurological and produced meticulous studies of the posterior commissure, the subcommissural region, muscle spindles and intramuscular sensory organs: her pioneer investigations of the process of myelination earned the award of the DSc of London University.

Lucas Keene was exceptionally endowed with powers of organisation and judicial assessment and was wholeheartedly committed to duty. These qualities gained early recognition and were inevitably empanelled in the general service of School and University. Without remission of departmental commitment she accordingly assumed and discharged with notable success an onerous burden of scholastic and administrative business which involved, during the Second World War, added responsibility for the School's migrant preclinical departments and the reconstruction of its bomb-devastated premises.

Her integrity and profound common sense fortified by her personal dignity and charm, redounded to her School's prestige and engendered wide extramural appreciation of her judgement and counsel. Her achievements and services were fittingly recognised by her election to the presidency of the School of Medicine (1956-74), of the Anatomical Society (1949-51) and of the Medical Women's Federation (1946-49), to vice-presidency of the Medical Protection Society (1929-77), to the livery of the Worshipful Society of Apothecaries (1952) and to the Fellowship of the Royal College of Surgeons of England (1956).

A. J. E. Cave